SPERM QUALITY AND REPRODUCTIVE HORMONES CONCENTRATIONS IN WHEAT (*TRITICUM AESTIVUM*) LECTIN-FED MALE PRE-PUBERTAL SPRAGUE-DAWLEY RATS

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ABSTRACT

Introduction: Unexplained infertility in Nigeria is on the increase with male factor accounting for about 50% of case of which 25% of cases are idiopathic. Evidence indicates that wheat lectin (Wheat Germ Agglutinin -WGA) induces damage to reproductive tissues and that may be a precursor of infertility in humans. **Objective:** This study was to determine the effects of WGA on sperm and reproductive hormones' parameters using experimental rat models. **Materials and Methods:** Forty-five male pre- pubertal Sprague-Dawley rats (40.89±9.40 g and 30-35 days old) were used for the study in three phases, represented by different time intervals of two, five and ten weeks, respectively. Each phase consisted of three groups of five rats each, (A, B, C), (D, E, F) and (G, H, I), respectively. T. *aestivum* lectin was extracted by ammonium sulfate precipitation (60%), purified by dialysis and powdered by lyophilization. The lectin fraction at 250 mg/kg was subsequently administered orally to the experimental rats daily for 2wk, 5wk and 10wk respectively. Equal dose of standard wheat germ lectin (WGL) also administered in parallel to the control rats. Animals in groups A, D and G served as negative control and received 10 ml/kg body weight of distilled water. All administrations were done orally. At the end of each experimental phase, rats were weighed and blood collected. They were sacrificed, epididymides and testes were collected, blood epididymides as well as testisticular tissues were processed for the measurement of sperm quality and reproductive hormones. **Results:** The administration of pure lectin for 2, 5 and 10 weeks caused significant ($P < 0.05$) decrease in sperm motility by 21.4%, 51.2% and 47.2%, respectively and increased abnormal morphplogy by 14.2%, 13.4% and 14.6% , respectively .The percentages of sperm count also significantly decreased by 46.9% and 63.35% in the 5 and 10 weeks groups respectively but remained unaffected in the two weeks group. Significantly ($P < 0.05$) higher levels of FSH, LH, Prolactin and low level of serum testosterone in a time dependent manner were also observed when compared to the negative control at $P< 0.05$. **Conclusion:** Results suggest that wheat lectin reduces sperm count, motility, and morphology and serum testosterone, suggesting its potentials to have a deleterious effect on the fertility of male pre-pubertal rats. Further evaluation of the reproductive consequences of wheat lectin consumption is recommended.

Keywords: *Wheat germ agglutinin, Sperm quality, Reproductive hormones, Testes, Pre-pubertal-rats.*

INTRODUCTION

Wheat is one of the most commonly consumed cereal grains worldwide as it makes up a substantial part of the human diet as well as livestock feed (Shewry, 2009). Since the lifting of the wheat ban in 1992, Nigeria has become a huge export market for *Triticum aestivum (*modern bread wheat or common wheat) due to the high demand for wheat flour for the production of bread, noodles, pasta, pastries, breakfast cereals etc. This has contributed to Nigeria's wheat market to be estimated at just under $1 billion in U.S. exports (GAIN, 2014). Of the 30 species

of wheat known, *Triticum aestivum* is the most widely cultivated in the world (Mullen *et al.*, 2002; Shewry, 2009).

Despite the various nutritional and health benefits associated with wheat consumption, the presence of anti-nutritional substances in wheat remains a cause for concern by nutritionists, public health scientists and clinicians worldwide (Karin & Leo, 2013;Vasconcelos & Oliveira, 2004; Yarden & Sliwkowski, 2001). Lectin also called wheat germ agglutinin (WGA) – is one of the anti-nutritional compounds in bread wheat (Pellegrina *et al.*, 2009). Lectins are simply defined as proteins which specifically bind (or crosslink) carbohydrates. Although lectins as carbohydrate binding proteins play a defensive role against pathogens in wheat like in other plant species, they also elicit pathological effects on animal and human cells ((Freed, 1985; Rüdiger and Gabius, 2001). These include haemmaglutination, intestinal mucosa damage and nutrient absoption impairment (Pusztai, 1993; Hamid & Masood, 2009). They have also been implicated in autoimmunity (Pellegrina *et al.*, 2009) and hormonal imbalances (Pusztai, 1993; Vasconcelos & Oliveira, 2004). The later suggest that wheat lectins may play a role in infertility in humans.

Infertility is a worldwide problem, affecting 8-15% of the couples in their reproductive age (Boivin., 2009; Puscheck and Woodad,2009). However, the incidence rate of infertility varies across the globe and Nigeria falls within the infertility belt of Africa (Akinloye &Truter, 2011). In Nigeria, cases of infertility have been reported to be on the increase with male factor accounting for about 50%. Nearly 25% cases of male infertility are idiopathic (Khalil *et al.*, 2012).Male infertility has been associated with a variety of factors including higher exposure risk to toxic chemicals such as agrochemicals, cadmium, lead and aflatoxins as well as the high prevalence of sexually transmitted infections. Other important risk factors include tobacco smoking, excessive alcohol intake, use of native medications and drugs, low socio-economic status and having multiple sexual partners amongst others (Akinloye and Truter, 2011; Abarikwu, 2013) . Studies have shown that nutrition play a vital role in fertility issues Gaskin *et al.*, 2012.

Available evidence reveals that male factor infertility has not been given its deserved attention in issues of reproductive health, Okonofua, 2005; Onyeka *et al.*, 2012.

Poor semen quality and reproductive hormones imbalances have been documented to contribute to reduced fertility among Nigerian men (Abarikwu, 2013). This study was therefore designed to determine the effects of wheat lectin (Wheat Germ Agglutinin) on sperm and reproductive hormones parameters using experimental rat models.

METHODS

Ethical guidelines

Experimental protocol was approved by the Ethical committee of the Department of Anatomy, University of Lagos, Nigeria for use of laboratory animals. The study was approved to be in compliance with the international guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding Principles in the Care and Use of Animals (World Medical Association & American Physiological Society, 2002). OECD guidelines were followed.

Collection and Authentication of Plant Material

Hard Red Spring Whole Wheat Kernels (HRSWWK) was purchased from a vendor at Mile 12 market, a local market in Lagos, Nigeria on the 6th of August, 2012.

The taxonomic identification was done by wheat breeders, Y. Yakubu and A. Mustapha at Lake Chad Research Institute, Maiduguri, Borno State, Nigeria. (germplasm number- CM33027-F-15M-500Y-OM-87B-OV) In addition, voucher specimens with accession number LUH 6150 were deposited in the herbarium of Botany Department of the University of Lagos, Nigeria for reference.

Reference Standard: Pure lectin (wheat germ agglutinin) was purchased from Bio-Research Products, Inc. North Liberty, Iowa, U.S.A. (Product Ref. WGL3P5). All other chemicals used were of analytical grade.

Preparation of Crude Wheat Lectin Fraction

HRSWWK were picked, washed, dried in a hot air oven at 40°C and weighed. The % dry weight was grinded with Thomas Wiley mill, Model ED-5, London to powder form. It was sieved with a standard mesh. Lectin extraction was achieved by salting out method using ammonium sulfate at 60% saturation as described by Kalaivani, *et al.,* (2012).

Lectin yield and protein concentration determination

Crude lectin extraction yield after lyophilization was determined according to Zhang *et al.,* (2007), while Bradford's reagent (Bradford, 1976) was used to quantify the total protein content in the lectin fraction and crude wheat fraction using bovine serum albumin (BSA) as the standard.

Haemagglutination assay

This was carried out according to the method described by Awoyinka and Dada, (2011) using formalinized erythrocyte preparation at 4% from chicken, human, goat ,rat and rabbit red blood cells in phosphate buffered saline (pH 7.2) in 2-fold serial dilutions for 30 min at 25^0C. Hemagglutination titer, defined as the reciprocal of the highest dilution exhibiting hemagglutination, specific activity was expressed as the number of hemagglutination units per mg protein.

Acute Toxicity Studies

Acute toxicity studies were conducted on both crude lectin fraction extract and the pure substance via oral and intraperitoneal routes to obtain the median lethal dose (LD_{50}) using the method of Miller and Tainter as described by Agbaje & Fageyinbo (2012).

Animal Management and lectin Administration

This study was carried out on forty-five healthy male prepubertal Sprague-Dawley rats (30-35days old) with mean body weight of 40.89±9.40g. The rats were obtained from the breeding stock of the National Institute of Medical Research (NIMR) Yaba, Lagos. After acclimatization with standard care for 7 days, the rats were randomized into three groups of 5 animals per group in three phases, (A, B, C) (D, E, F) and (G, H, I), respectively.

Phase one (short term experiment) lasted for two weeks and was conducted on rats in groups A, B and C. The rats in Group A served as the negative control and received 10 ml/kg body weight of distilled water (vehicle for the extract). Group B served as the positive control and received 250 mg/kg body weight of standard WGA . Group C received 250 mg/kg body weight of crude lectin fraction.

Phase Two (middle term experiment) lasted for five weeks and was conducted on rats in groups D, E and F where D served as the negative control and received 10 ml/kg body weight of distilled water. Group E served as the positive control and received 250 mg/kg body weight of standard WGA. Group F received 250 mg/kg body weight of crude lectin fraction.

Phase Three (Long term experiment) lasted for ten weeks and was conducted on rats in groups G, H and I. The rats in group G served as the negative control and received 10 ml/kg body weight of distilled water. Group H served as the positive control and received 250 mg/kg body weight of standard WGA. Group I received 250 mg/kg body weight of crude lectin fraction. All

administrations were done daily orally through intra-gastric cannula between 08:00 and 08:45am.

Sample Collection and Tissue Preparation for Bioassays

After the last treatment for each study phase, the rats were fasted overnight and weighed the following morning. Blood samples were collected into plain tubes,the animals were then sacrificed after a light anaesthesia, followed by excision of caudal epididymides, testes, liver, heart, kidney, brain, spleen and pancreas pancreas for sperm function, hormonal, histological and biochemical analyses.

Sperm Function Analyses : This entailed the determination of sperm motility, morphology and counts as described by Cheng *et al.*, (2006), Akunna *et al*., (2013) and
Ali & Adel, (2013).

The testicular tissue was homogenized and used for testosterone assay, while the serum samples were used for the determination of Testosterone (T), Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH) and Prolactin (P) concentrations were determined using standard ELISA techniques and ELISA kits with catalogue numbers 2095Z, 4224Z,4225Z and 4226Z, respectively.

Statistical Analysis

SPSS) version 20.0 software was used to analyze the data obtained. Data were expressed as Mean X ± Standard Deviation (SD).

Differences between variables with normal distribution were analyzed using one-way Analysis of Variance (ANOVA) test and between groups were assessed using non-parametric student's T-test. A P-value of $P<0.05$ was considered statistically significant.

RESULTS

Acute Toxicity Test

No mortality was observed from oral feeding of up to 5000 mg/kg body weight of the crude lectin extract, whereas, intra-peritoneal dosing of resulted in an
LD_{50} of 446.7 mg/kg body weight. Also, oral feeding of up to 5000 mg/kg body weight of pure lectin (WGA), resulted in an LD_{50} was interpolated as 1778.3 mg/kg body weight, whereas, intra-peritoneal dosing of pure wheat lectin (WGA) recorded an LD_{50} of 72.4 mg/kg body weight.

Figure A shows a statistically significant decline in sperm motility in Groups B,E and H administered pure lectin (WGA) for 2, 5, and 10 weeks, respectively with mean values of 29.20 ± 12.21, 31.20 ± 12.76 and 47.60 ± 4.04 when compared with groups A, (50.60 ± 5.22) ,D(82.40 ± 6.07) and G (94.80 ± 7.98) , respectively at $P<0.05$.

Similarly, the percentage of sperm morphological abnormalities (headless, round head, banana head, bent neck, bent tail, rudimentary tail) were significantly higher in groups B, E and H administered pure lectin (WGA) for 2, 5, and 10 weeks, respectively with mean values 35.80 ± 3.35; 32. 60 ± 5.03 and 24.80 ± 8.26 respectively when compared with negative control values of group A, D and G, respectively with mean values of 21.60 ± 6.95; 19.20 ± 6.91 and 9.40±3.13, respectively at $P<0.05$.No significant differences were observed in sperm morphology between the negative control groups (A, D and G) and the groups administered 250mg of crude lectin for 2, 5, and 10 weeks respectively at $P>0.05$.

Epididymal sperm count (ESC) was significantly declined in the groups(E,F,H and I) administered 250 mg/kg body weight of pure as well as crude lectin fraction for 5 and 10 weeks,

respectively when compared to the negative control groups(D and G) whereas no changes were observed in the 2 weeks groups(B and C) given equal doses of pure and crude lectin fraction. The mean serum Luteinizing Hormone (LH), Prolactin (P) and intra-testicular testosterone (IT) levels were significantly increased in animals (group B) receiving 250 mg/kg body weight of crude lectin for 2 weeks when compared with the values of the negative control group(A) at $P<0.05$.Administration of 250 mg/kg body weight of crude lectin for 5 and 10 weeks also resulted in significant increases in serum FSH, LH, Prolactin (P) and Testosterone (T) levels in groups (F and I) when compared to the negative control values in groups D and G, respectively at $P< 0.05$.There was no significant change in the level of intra-testicular testosterone after 5 weeks of administration of crude and pure lectin to animals in groups E and F, however, intra-testicular testosterone levels were significantly increased in groups H and I after 10 weeks of administration. FSH and P levels were also significantly increased in pure lectin group H after 10weeks of administration when compared to the negative group G at $P< 0.05$ (Figure B).

DISCUSSION

Sperm parameters such as count, motility and morphology are key indices of male fertility, and they are the prime markers in testicular spermatogenesis and epididymal maturation (Rolland *et al*., 2013). Decreased sperm count, motility and increased abnormal sperm cells are usually associated with decreased fertility rate (Rolland *et al*., 2013).

When 250 mg/kg body weight of pure and crude wheat lectin were administered to pre-pubertal rats for two, five and ten weeks, respectively, the epididymal sperm count and sperm motility declined significantly compared to the negative control groups while morphological abnormalities of the sperm cells like clumping, headless, round head, banana head, bent neck, bent tail, rudimentary tail were significantly increased in the groups that received 250 mg/kg body weight of pure lectin. Our findings suggest that oral administration of wheat lectin reduces sperm quality indicating that high consumption of modern wheat-based diet which contains anti-nutrients predisposes to decreased fertility. Our findings are in accordance with the report of Oyedeji *et al*., 2013, who carried out a study on a widely consumed nutritious plant, bitter-leaf, they reported significant decreases in sperm motility and count relative to control following the administration of different doses of the methalonic extract of the *Vernonia Amygdalina* (bitter-leaf .

Anti-nutrients in plant foods are responsible for deleterious effects related to the absorption of nutrients and micronutrients. Anti-nutritional factors have been reported to diminish animal productivity but may also cause toxicity during periods of scarcity or confinement when the feed rich in these substances is consumed by animals in large quantities (Kumar, 1992). In males, reproductive function in young animals appears to be more susceptible to dietary restrictions of energy and protein than in adult and may lead to permanent histological changes at the level of the testis (Brown, 1994). The clumping together of sperm cells seen in this study could be as a result of agglutination caused by lectin.

Gonadotropins (FSH, LH) and testosterone are the prime regulators of germ cell development. Abnormal spermatogenesis is often associated with altered serum gonadotropins and testosterone (Cheng *et al*., 2010). Our present study demonstrated elevated LH and FSH concentrations in serum with concomitant reduced level of serum testosterone following the administration of 250 mg/kg body of crude lectin indicating primary testicular failure. Serum LH level rises when testosterone production falls because the negative feedback effects of testosterone on Gonodotropins releasing Hormone (GnRH) secretion are reduced. A reduced T level has been

adduced for the significant reduction in the epididymal and testicular sperm number and therefore daily sperm production in oligospermic males (Elbeticha and Da‟as, 2003).

Prolactin on the other hand has long been known to be a hormone responsible for mammary gland development and lactation in females. Hyperprolactinemia has been documented to causes infertility in about 11% of oligospermic males (Masud *et al.*,2007). This study showed a higher serum levels of prolactin in the in rats administered 250 mg/kg body weight of crude lectin for two,five and ten weeks, respectively when compared to the control proving a role of prolactin in gametogenesis, which is independent of gonadotrophins(Palomino & Herrera 1990).The increase in the prolactin levels of the pure lectin group in the five and ten weeks groups but not the 2 weeks group could suggest that the increase is time dependent, also the increase in crude lectin group from two weeks could suggest that other anti-nutrints present in the extract could also contribute to the elevated prolactin level. Several studies suggest that hyperprolactinemia has a definite role in male infertility, and is one of the reversible causes of infertility (Singh *et al.*,2011; Ciccarelli *et al.*,2005). Hyperprolactinemia also directly influences spermatogenesis and steroidogenesis by acting on prolactin receptors present in Sertoli cells and Leydig cells in testes, and produces primary hypogonadism and infertility (Masud *et al.*, 2007). In reality, prolactin's role is so vast that its complexity is incalculable, having been found to have approximately 300 separate actions in vertebrates (Bole-Feysot *et al*., 1998; Fanciulli *et al.*,2004). Any disruption therefore of its normal function or concentrations would have a wide range of downstream and potentially unpredictable adverse effects. The fact that wheat can act like an endocrine disruptor is not well established, but a provocative human trial confirms for the first time that one of the adverse effects of wheat consumption includes a disruption of the levels of prolactin Delvecchio *et al.*,2014. The researchers hypothesized that in newly diagnosed celiac disease (CD) patients, an increase in prolactin may be due to increased production of inflammatory cytokines, such as IL-1 and IL-6, which are typically elevated in CD patients not on a gluten-free diet, and decreased in those who are not eating gluten. They state, "The PRL reduction after 6 months of GFD may be likely due to the reduction of the inflammatory cytokine."

CONCLUSION

Results suggest that wheat lectin reduces sperm count, motility, and morphology and serum testosterone, and therefore may have a deleterious effect on the fertility of male pre-pubertal rats. It could therefore be suggested that wheat lectin is a hormonal disruptor. Further evaluation of the reproductive consequences of wheat lectin consumption is recommended. Hormonal assessment of the serum prolactin level should be included during evaluation of male infertility.

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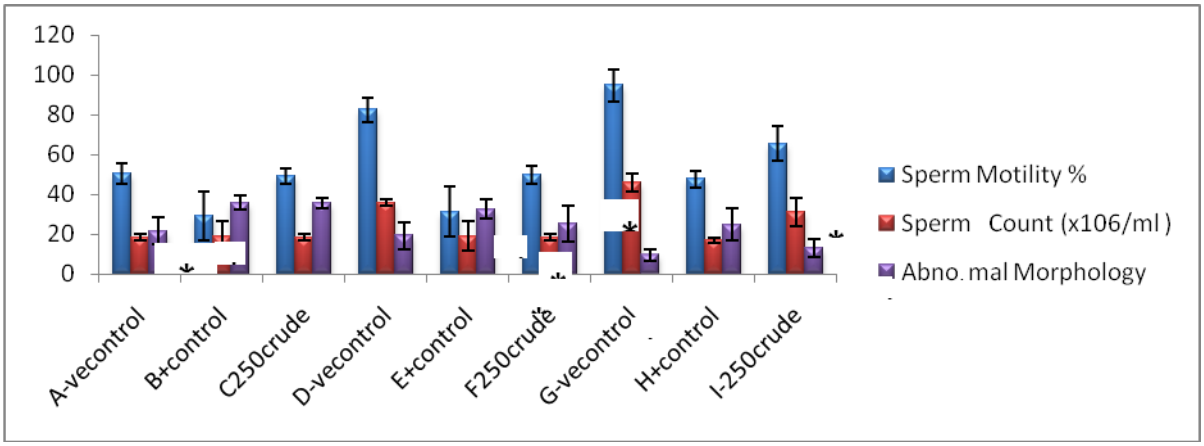

Figure A: Sperm motility, count and abnormal morphology after two, five and ten weeks of administration

Values are expressed as mean ± SD. Of 5 rats; * represents significant difference when compared with A, D and G ($P < 0.05$).

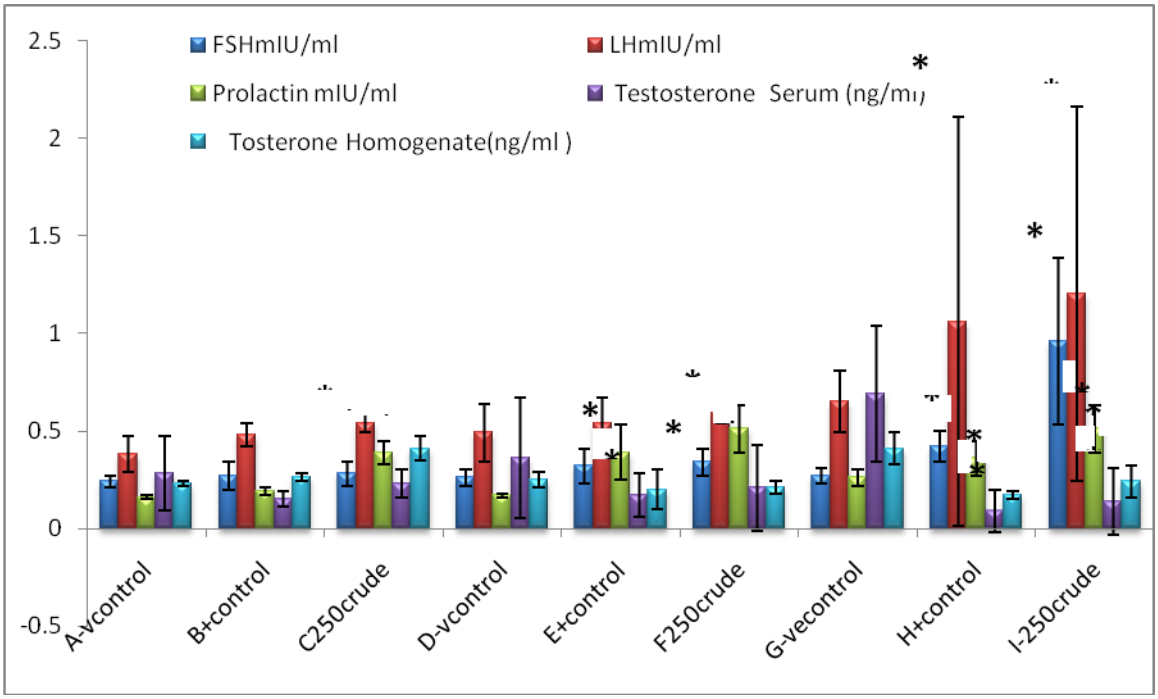

Figure B: Reproductive hormones levels after two, five and ten weeks of administration Values are expressed as mean ± SD. (n=5); * represents significant differences when compared with A, D and G ($P < 0.05$).

C-REACTIVE PROTEIN AS A LIKELY BIOMARKER FOR UNCOMPLICATED MALARIA

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ABSTRACT

Background: Malaria is public health disease that presents with symptoms that are similar to other bacterial and viral infections that stimulates inflammation. C-reactive protein (CRP) is one of the markers of inflammation which is identified to increase with high fever. The connection between malaria and C-reactive protein is still emerging. Therefore, understanding the relationship between CRP and malaria parasitaemia is likely to guide its development as a possible biomarker for malaria. **Objective**: To correlate CRP and malaria parasitaemia in patients presenting with uncomplicated malaria in Lagos, Nigeria. **Methods**: This was a cross-sectional study conducted several health facilities in Lagos State, Nigeria. The subsets of patients (100 patients) used for the study were selected from participants who presented with fever or with history of fever in the last 24hours. Patients presenting symptoms were captured in a designed case report form (CRF), blood smears done for malaria microscopy and plasma obtained from the 4mls of venous blood that was collected from each patient. Absolute white blood cell was also determined for each patient. The separated plasma was used to run the CRP latex agglutination qualitative and semi quantification assay. **Result**: This exploratory study recorded a positive correlation between CRP and parasitaemia which was statistically significant (Spearman's rho correlation coefficient= 0.845, P<0.001 and Pearson Correlation= 0.795, P<0.001). Also, the highest level of CRP range (49->96mg/l) was seen in individuals with higher parasitaemia which ranges from 244.4-21615.5. **Conclusion**: High parasitaemia correlated positively with high CRP level though the overall correlation of all parasitaemia was weak. This is suggestive that CRP could be explored for its possible potential as a biomarker for malaria using a larger sample.

Keywords: Malaria Parasitaemia, CRP, Biomarker, High malaria parasitaemia, Malaria diagnosis.

INTRODUCTION

Inflammation is a part of a complex biological response of the immune system to disease causing organisms, damaged cells or irritants which is grouped by five signs namely redness (rubor), swelling (tumor), heat (calor), pain (dolor) and loss of function (functio laesa) (Punchard *et al.*, 2004). Inflammation is a mechanism that helps the body get rid of foreign matter also known as non-self and dispose cells that are not useful, which could result in healing.

The immune system is a system that helps to protect the body from possible infectious agents as well as causing occasional harmful effects (Harpaz *et al.*, 1992). It is known to separate the self from non-self. The immune system is composed of two major subdivisions, the innate or non-specific immune system and the adaptive or specific immune system (Perlmann and Troye-Blomberg, 2002, Mayer *et al.*, 2011). During malaria infection, the immune system reacts bringing about inflammation. As a result of these activities, certain proteins are formed to help fight off the infection, one of such protein is the CRP (Naik and Voller, 1984; Ansar *et al.*, 2009; Paul *et al.*, 2014).

CRP has been identified as a biomarker of inflammation (Dharmapalan and Yewale, 2012), and a prognostic marker in malaria (Paul *et al.,* 2014) which is known to induce adhesion molecule expression in human endothelial cells (Pasceri *et al.,* 2000), ligand binding, activation of complement (Mold *et al.,* 1999; Ansar, 2009; Dharmapalam and Yewale, 2012), opsonization and antigen presenting (Pepys and Hirschfield, 2003; Hela *et al.,* 2012), protection against pre-erythrocytic stages of malaria (Pied *et al.,*1989), and could increase tolerance to malaria (Hurt *et al.,* 1994). It has also been identified that CRP and the classical component acts together to promote noninflammatory clearance of apoptotic cells (Gershov *et al.,* 2000). These functionalities help in the prompt identification and clearance of the parasite by the immune system .

C reactive protein (CRP) level has been said to increases with the severity of malaria (Kremsner *et al.,* 1997; Bouree *et al.,* 2002; Nahrevanian *et al.,* 2008; Dongmo *et al.,* 2011) and could be used as a diagnostic and management tool in malaria holoendermic areas like Nigeria to reduce disease burden (Amah *et al.,* 2011; Andrade *et al.,*2011*).* CRP has also been identified to have adverse patholovical effects like the clearance of RBC which results in severe anaemia (Ansar *et al.,* 2006; Ansar *et al.,* 2009; Isrealsson *et al.,* 2009), increasing susceptibility to *Plasmodium falciparum* malaria among Sudanese donors (Giha *et al.,* 2010) and, playing a part in the expression of experimental cerebral malaria (Szalai *et al.,* 2014). Malaria is a parasitic disease of importance in Nigeria and the better understanding of the "malaria-human immune" relationship will help the better management of the disease. Therefore, the aim of this exploratory study was to determine the relationship between CRP and malaria in patients with uncomplicated malaria in Lagos, Nigeria.

METHODS

Study Area and Participant Recruitment:

Ethical approval was received from the ethics committee, College of Medicine, University of Lagos. This study was carried out in Lagos state situated in the south-west region of Nigeria. Sample population was gotten from four study sites which include Regina Mundi Catholic Hospital, Mushin, Randle General Hospital, Surulere, Igando General Hospital, Igando, Badagary General Hospital, Badagry. A total number of 100 individuals participated in this study. Consent forms where used to obtain approval from the study participants. Individuals presenting with fever within the last 48hours that came to the hospital laboratory of the sites and agree to the terms in the consent forms and signed them were included in this study while, individuals that do not give their consent, and as such do not sign the consent forms were not included in this study.

Sample Collection:

Blood samples were collected from patients presenting with fever or those with history of fever who were suspected to have malaria. About 2ml of blood samples were collected into EDTA containers. A total number of 100 individuals participated in this study out of which 12 were from Mushin, 25 from Badagry, 25 from Igando and 38 from Randle. The samples collected were taken to the Tropical Disease Research Laboratory, College of Medicine University of Lagos Lagos, Idiaraba, where they were processed.

Blood Film Preparation and Staining: Thick and thin blood films were made and slides were stained following the WHO standard procedure for the preparation of blood films and staining. The patient's absolute white blood cell count was used to determine the patient's parasite load per micro-litre of blood. The microscopy was also done following the WHO standard and there was two blinded independent reading of the slides to minimize errors.

SEPARATION OF PLASMA

The blood samples collected in the EDTA bottles were spurned at 4000 revolution per minute for ten minutes. The plasma was separated from the EDTA container into cryogenic vials. The separated plasma was used for the serological of CRP.

The Biotec Cambridge CRP latex test kit for 100 tests was used to determine the presence of CRP in the separated sera following the manufacturer's instructions.

Semi-quantitative Method: A serial dilution of the plasma samples were done and calculated for. Using normal saline, 1 µl of saline was placed in about 5 tubes and another 1 µl of sample was placed in the first tube which is mixed properly and 1 µλ of mixed sample with saline is taken using a new pipette tip and mixed in the next tube containing normal saline and mixed properly; thus a serial dilution was done on each positive sample (Table 1). A drop (40µλ) of reagent is placed in the circle of the slide and the 50µλ of sample prepared via serial dilution is added, the reagent and the serum is spread round the circle and tilted backwards and forward approximately once every 2seconds for 2minutes. This is done to determine the highest dilution that will show reaction. Once this is determined, the estimated level of CRP in the sample is calculated and recorded.

RESULTS

100 participants were recruited and tested for significant level of C-reactive protein (CRP >6); the malaria parasite density was also determined. This study included 61 females and 39 male participants. Individuals tested included those with fever (>37.5^{0}C) and those with history of fever in the last 48 hours but presented with normal temperature at the time of sample collection (<37.5^{0}C). Of the 100 participants, 75 presented with fever (temperature >37.5^{0}C) at the time of sample collection; 25 presented with normal temperature at the time of sample collection. A total of 13 microscopy positive results were tested for CRP, 8 had CRP level of 0-95mg/L while 5 had CRP level of 96-384mg/L (Table 2).

CRP is associated with an increase in temperature in individuals with fever (88.9%) when compared to those without fever (11.1%). The relationship between CRP level and parasite density using the Spearman's rho correlation coefficient= 0.845, P<0.001 and Pearson Correlation= 0.795, P<0.001 (Figure 1). The higher level of CRP (96-384mg/l) is seen in individuals with high parasitaemia which ranges from 244.4 to 21615.5 P/µl of blood, while those with low parasite density had lower concentration of CRP (Table 3).

DISCUSSION

Of the 13 microscopy positive tested for CRP, 8 had CRP level of 0-95mg/L while 5 had CRP level of 96-384mg/L (Table 2). This indicates that CRP is a marker associated with malaria since there was a significant increase in the level of CRP in participants with higher parasitaemia when compared with those with lower parasitaemia as identified in other studies (Haghighi 1969; Naik and Voller 1984; Hurt *et al.,* 1994; Israelsson *et al.,* 2009; Kutsuna *et al.,* 2014).

This study further highlighted that CRP is associated with an increase in temperature as seen in the number of individuals without fever (<37.5^{0}C) at the point of sample collection that had high level of CRP between 96-384 mg/L (11.1%) when compared to those who presented with fever (>37.5^{0}C) at the point of sample collection (88.9%), thus identifying CRP as a marker of inflammation associated with fever since fever is a sign of systemic inflammation which is supported in a study carried out by Dharmapalam and Yewale (2012) .

The study also showed a strong positive relationship between CRP level and parasite density (Spearman's rho correlation coefficient= 0.845, P<0.001 and Pearson Correlation= 0.795,

P<0.001). It was noted that the highest level of CRP (96-384mg/l) is seen in individuals with higher parasitaemia which ranges from 244.4 to 21615.5 parasites per microlitre of blood, while those with low parasite density had lower concentration of CRP from 22-23 parasites per microliter of blood (Table 3). This is supported by some studies which identified that the level of C-reactive protein increases with the severity of malaria infection (Kremsner *et al.,* 1997; Bouree *et al.,* 2002; Nahrevanian *et al.,* 2008; Dongmo *et al.,* 2011). A study by Kutsuna and others (2014) also recorded that the level of CRP increases with increased parasitaemia which could be used for diagnostic basis to differentiate malaria fever from non-malaria fever like Dengue fever in areas where other tests are not available.

CRP can be used as a prognostic marker since its concentration in human sera reduces with reduction in parasitaemia and increases with increased parasitaemia; this is supported by other studies (Naik and Voller, 1984; Paul *et al*., 2014) which affirmed that CRP can be used as an effective biomarker in assessing malaria severity and could also be used as a follow-up test for malaria patients to track recovery progress.

CRP may also have an important role to play in the immune response to malaria since there is a possibility the marker is involved in the clearance of the parasite or the pathogenesis of the disease due to its high concentration during high parasitaemia and lower concentration in cases of low parasitaemia. This is supported by other studies who stated that CRP may play an important role in immune response to malaria through the process of inhibiting dendritic cells, neutrophils or complement regulatory proteins resulting in the clearance of RBC which results in severe anaemia (Ansar *et al.,* 2006; Ansar *et al.,* 2009; Isrealsson *et al.,* 2009). Also C-reactive protein can bring about systemic autoimmunity through binding to apoptotic cells and protecting the cells from terminal complement Components, thereby sustaining an anti-inflammatory innate immune response (Gershov *et al.,* 2000). Some other studies recorded a strong association between increase malaria susceptibility and presence of CRP-286 A-allele (Giha *et al.,* 2010) and, the expression of experimental cerebral malaria (a sign of severe malaria) as being promoted by CRP (Szalai *et al.,* 2014). Another study in Ghana suggested that CRP levels are positively related to immune responsiveness and malaria parasitaemia (Eriksson *et al.,* 2013). Furthermore, Hurt and others (1994) stated that CRP can be used to track the acquisition of tolerance to malaria thus implying that it plays protective function in malaria patients. Also, another study further stated that CRP could be useful for malaria immunoepidemiology; however, it was not stated if CRP is beneficial or detrimental (Nahrevian *et al.,* 2008).

CONCLUSION

In this exploratory study, it has been shown that the concentration of C-reactive protein in sera increases with the increase in malaria parasite density in patients presenting with uncomplicated malaria in Lagos, Nigeria. This therefore, might indicate that CRP is associated with the functionality of parasite clearance in malaria patients since the protein level is seen to increase with an increase in parasite density or could also be involved in the disease pathogenesis of which further studies on the immunological role of CRP will help to determine its precise immunological function. These observations could be applied in therapeutic purposes to harness other natural therapeutic measures aside from drug administration.

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Table 1: Serial dilution procedure and calculation

Dilutions	½	¼	$^1/_8$	$^1/_{16}$	$^1/_{32}$	$^1/_{64}$	$^1/_{128}$
Sample Serum	100µl	-	-	-	-	-	-
Saline	100µl	100µl	100µl	100µl	100µl	100µl	100µl
Volume of Sample used on test slide	50µl	50µl	50µl	50µl	50µl	50µl	50µl
6 x Titer	6 x 2	6 x 4	6 x 8	6 x 16	6 x 32	6 x 64	6x128
Mg/ml	**12**	**24**	**48**	**96**	**192**	**384**	**768**

Table 2. Baseline characteristics of the study population

Variables	CRP (0-95 mg/L) (n=73)	CRP (96-384 mg/L) (n=27)	P value
Age (Years)			
Mean ± SD	31.01 ± 16.01	22.30 ± 13.4	0.013
Sex			
Male	24 (32.9%)	15 (55.6%)	0.039
Female	49 (67.1)	12 (44.4%)	
Malaria			
Negative	65 (89%)	22 (81.5%)	0.318
Positive	8 (11%)	5 (18.5%)	
Febrile (Fever)			
No	22 (30.6)	3 (11.1)	0.047
Yes	50 (69.4)	24 (88.9)	
CRP			
Geometric mean	0	137.52	<0.001
Median (Range)	0 (0-48)	96 (96-384)	<0.001

Note: N = number of samples. SD = standard deviation. CRP = C-reactive protein (mg/L). P values were based on Pearson Chi-Squared test or Exact Chi-Square for categorical variables and ANOVA for the comparison of the mean of the continuous variables.

Table 3. CRP concentration compared with malaria parasitaemia

CRP Concentration (g/dl)	Malaria Parasitaemia (P/ml)
6-12	28
13-24	33
25-48	Nil
49-96	244.4-3017.2
>96	1560-21615.5

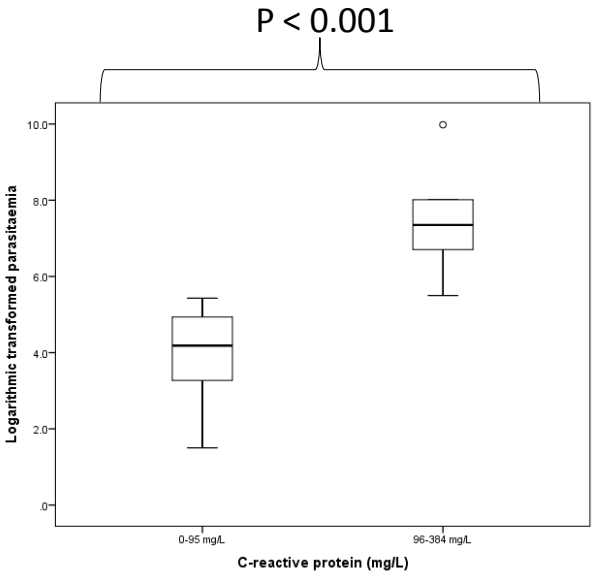

Figure 1. Logarithmic transformed parasitaemia level with CRP (mg/L). The boxes illustrate the total observations equivalent to the first quartile and the third quartile. The median is represented by the horizontal line. The outlier is shown as a circle point.

HEPATOPROTECTIVE AND THERAPEUTIC ACTIONS OF LIVER GEN® ON CARBON TETRACHLORIDE INDUCED LIVER DAMAGE IN FEMALE WISTAR ALBINO RATS

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ABSTRACT
Background: *Liver gen*® is a Chinese herbal product with claims of potency against variety of diseased conditions and it enjoys considerable patronage in Nigerian society by people plagued with ill health. **Objective**: The study was undertaken to evaluate the hepatoprotective effect, therapeutic actions and acute toxicity of *Liver gen*® on carbon tetrachloride induced liver damage in female Wistar albino rats. **Materials and methods**: Acute toxicity study was carried out in albino mice and the LD_{50} for *Liver gen*® determined. Albino rats weighing 180-200g were divided into four groups. Hepatic injury in rats was induced in groups I – III by the administration of equal mixture of Carbon tetrachloride (CCL_4) and olive oil (50% v/v, 1ml/kg body weight intraperitoneally) every 72 hours for 10 days. Group I (negative control) was not treated while groups II and III were subsequently treated with *liver gen* administered orally at a dose of 28.6mg/kg and 57.2mg/kg body weight respectively for 14 days. Group IV (normal control) received distilled water throughout the study period. After the treatment period the animals were sacrificed, serum was obtained from the blood while liver, brain and kidney were excised. Employing standard biochemical assay protocols, hepato-specific biomarkers alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phospatase (ALP), renal functions test (urea and creatinine), hematological indices such as haemoglobin concentration(Hb), pack cell volume (PCV) and white blood cell count (WBC) were determined in the serum. Antioxidant status- superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), malondialdehyde (MDA) and histopathological features were assessed in the rat tissues. Heavy metals' contamination was also assessed in the product using atomic absorption spectroscopy. **Results**: Levels of liver function marker enzymes such as alanine amino transferase (ALT) decreased ($p<0.05$) significantly in CCl_4 treated groups, urea concentration reduced significantly in the CCl_4 while SOD and CAT showed no significant ($p<0.05$) difference in the treated rats compared with the control. Glutathione-s-transferase activity reduced ($p<0.05$) significantly in the CCl_4 treated group and increased on treatment with the Liver gen®. The effect of Liver gen® on the PCV and WBC was dose dependent. No mortality was recorded in the acute toxicity study at the highest dose of 20,000mg/kg. Quantitative analysis of the heavy metals present in Liver gen showed a higher percentage of copper compared to cobalt, lead, zinc, iron, nickel, manganese and chromium ( ). Histopathological examinations of the liver sections confirmed the biochemical results and indicated that CCl_4 induced severe histological lesions in the hepatic, renal and brain tissues which was ameliorated by Liver gen® administration. The present observation suggested that the treatment with Liver gen® enhanced recovery for CCl_4 induced hepatic damage. It could therefore be a good natural food supplement capable of reducing oxidative stress and serve as an hepatoprotective agent in particular.

***Keywords**: Hepatoprotective, Aminotranferases, Liver gen®, Antioxidant.*

INTRODUCTION

Liver is the largest single organ in the human body and the site for intense metabolism. It has a wide range of functions which includes processing and storage of many of the absorbed nutrients

from the intestine which are necessary for body functions. The major functions include carbohydrate, fat and protein metabolism; it also secrets bile into the intestine which enables it to absorb nutrients (1). The liver also detoxifies, synthesizes protein and also produces bio-chemicals that are needed for digestion. The importance of liver is underscored by general reference to other tissues as "extra hepatic tissues"

The liver is the first organ to encounter ingested nutrients, drugs and environmental toxicants that can enter the hepatic portal vein from the digestive system and the liver function can be detrimentally altered by injury resulting from chronic exposure to toxicants (2). The liver highly specialized tissues regulate a wide variety of biochemical reactions which includes the synthesis and breakdown of small and complex molecules which are necessary for normal vital functions (3).

Liver gen® is a product of the green world, a transnational company that engages in scientific research and development, manufacturing of pharmaceuticals, herbal products and herbal cosmetics. This product, the producer claimed it's a detoxifier; protects the liver from damaging toxins and also improves circulation of the blood flow to the liver.

Carbon tetrachloride is one of the most potent hepatotoxins that are widely used in scientific research to evaluate the hepatoprotective agents (4). Exposure to high concentrations of CCL_4 can affect the central nervous system, degenerate the liver (5), kidney (6) and may also result in coma after prolonged exposure and even death (7).

The study was aimed at verifying the claims by Green World Pharmaceuticals on the hepato-protective and regenerative activity of Liver gen® against carbon tetra chloride induced liver damage in rats and to investigate its safety for human consumption by ascertaining its acute toxicity in albino mice (determination of lethal dose of *Liver gen*®**)** and quantification of heavy metals present.

METHODS

Animals: Adult female albino rats (180-200g) were purchased from the animal house of College of Medicine of the University of Lagos, idi-araba, Lagos. They were housed five animals per cage in animal cages under standard conditions of temperature, relative humidity 12h light and 12h dark cycle and they were given food and water *ad libitum*

Chemicals: *Liver gen*® was purchased from World (Tianjin) Nutrition & Health Food Co., Ltd., China while Carbon tetrachloride was obtained from Sigma-Aldrich, United Kingdom. All other chemicals and reagents used in this study were of analytical grades

Acute Toxicity Study: Acute toxicity of the drug was evaluated by the method of Turner (8). Twenty albino mice divided into four groups of five mice per group were employed for the study. Group 1 which served as control received normal saline. Groups 2, 3 and 4 were administered 20000, 15000 and 10000 mg of *Liver gen*® / kg body weight respectively. Before the administration, feed was removed but water and other conditions were maintained. After the administration the animals were monitored for 4hrs in order to check their movement and death. They were also monitored for 24hrs later for mortality rate in percentage and prohibit taken. Heavy metals present in Liver gen were determined using atomic absorption spectrometry.

Experimental Animals: The animals were randomly distributed into four groups of five animals each. Hepatic injury in rats was induced in groups I – III by the administration of equal mixture of Carbon tetrachloride (CCL_4) and olive oil (50% v/v, 1ml/kg body weight intraperitoneally) every 72 hours for 10 days. Group I (negative control) was not treated while groups II and III were subsequently treated with *liver gen* administered orally at a dose of 28.6mg/kg and 57.2mg/kg body weight respectively for 14 days.. Group IV (normal control) received distilled

water throughout the study period. Liver damage was monitored by quantifying the raised marker enzymes.

Tissue Sample Collection and Preparation: At the end of the experiment, rats were sacrificed by cervical decapitation and blood samples were collected into clean, dry heparinised tubes and EDTA bottle. The serum was separated for biochemical analysis. Liver marker enzymes-Aspartate amino transferase (AST), Alanine amino transferase (ALT) and Alkaline phosphatase (ALP) were determined (9), urea and creatinine concentrations were evaluated while hematological indices (Hb, PVC, WBC) and oxidative stress biomarkers (SOD, CAT, GSH and MDA were also evaluated (10). The liver, brain and kidney tissues were excised and kept in 10% buffered formalin for histopathological studies.

Statistical Analysis: Data were presented as Mean ± standard deviation (SD) and analyzed using SPSS Version 17.0. Criterion for statistical significance was set at $p<0.05$.

RESULTS

Acute Toxicity Test

No mortality was observed when *Liver gen*® was administered orally at 10,000, 15,000 and 20,000mg/kg body weight.

Effects on Liver Function

Liver gen ameliorated the damages caused in rat liver by CCl_4 induced hepatoxicity in a dose dependent manner as the serum activities of ALT, AST and ALP were reduced in the treated experimental animals compared to negative control but comparable to normal control (40.4±6.26, 132.7±19.96 and 223.0±75.1 vs. 56.5±15.1, 152.6±29.5 and 296.5±23.6) Table 1.

Effects on Serum metabolites

There was no significance change in urea and creatinine levels ($p<0.05$) in all rats administered Liver gen when compared with the control (Table 1).

Effects on Haematological Indices

White blood cells and Pack cell volume decreased significantly ($p<0.05$) in the *Liver gen* treated groups compared to the normal control but no change to the Hb. The effects could largely be attributable to the effects of CCl_4 on these parameters (Table 2).

Effects on Antioxidant Parameters

Reduced glutathione**,** glutathione-S- transferase, superoxide dismutase and catalase activities increased in dose dependent manner in the treated group compared to the negative control and was similar to the normal control (1.83±0.15, 0.99±0.11, 1.3e-4±6.3e-6, and 0.04±0.05 vs 1.69±0.24, 0.74±0.12, 2.5e-5±1.3e-4 and 0.35±0.60). Malondialdehyde level was constant in both the treated and control groups (Table 3).

Effects on brain, liver and kidney histology

The photomicrographs of liver and kidney tissues of rats administered low and high doses of liver gen and the negative control administered CCl4 for 14 days at x100 magnification are presented in plates 1-4. Histological features of extensive hepatocellular degeneration and necrosis, fatty changes, inflammatory cell infiltration, congestion, and sinusoidal dilatation. The hepatocytes also showed vacuolar degeneration.

DISCUSSION

This study evaluated the protective ability of *Liver gen*® on liver damage induced by carbon tetrachloride. One of the most sensitive and dramatic indicators of hepatocyte injury is the release of intracellular enzymes such as amino transferases and serum alkaline phosphatase in blood circulation. This was observed in this study after CCl_4 administration. The elevated activities of these enzymes are indicative of cellular leakage and loss of functional integrity of the cell membranes in liver (11; 12).

In this study, the activities of AST and ALP were not altered, but there was a significant increase in the activity of ALT in the negative control compared with groups treated with *Liver gen*®

The body counters the effect of free radicals through endowed antioxidants. These antioxidants are produced either endogenously or received from exogenous sources and include enzymes like superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase. Other compounds with antioxidant activity include glutathione, flavonoids (13) which protect cells against oxidative stress (14). The reduced glutathione level decreased ($p<0.05$) in the CCl_4 induced group when compared to the treated group, while superoxide dismutase (SOD) and catalase (CAT) activities were unchanged in the treated groups and the controls. The Glutathione-S-transferase level reduced ($p<0.05$) in the CCl_4 induced group when compared to the treated groups. The lipid peroxidation level increased in the CCl_4 induced group, but decreased significantly in the treatment groups ($p<0.05$). Lipid peroxidation increased in the low *Liver gen*® dose group which indicates that it has free radical scavenging activity in the animal because at a higher dose the lipid peroxidation level decreased.

liver gen® administration to the rats did not alter the hematological parameters of the albino rats as there were no significant changes in these parameters between the control and the test groups (Table 3). The packed cell volume and the white blood counts showed that there was a significant decrease in the treated groups compared to the control group. Liver gen administration decreased packed cell volume (PCV) and white blood cell (WBC) in a dose dependent manner, such that an increase in the dosage of the drug caused a decrease in the PCV and WBC. It could be that the drug has an immunity lowering and blood cell destructive effects on the rats (Table 3).

The histological results from this study suggest biochemical changes in the hepatic tissues similar to earlier report (15). The acute hepatotoxic effects induced by CCl_4 administration were confirmed by histopathological studies which revealed extensive hepatocellular degeneration and necrosis, fatty changes, inflammatory cell infiltration, congestion, and sinusoidal dilatation. The hepatocytes also showed vacuolar degeneration. The treatment of the rats with liver gen protected the liver from damage by CCl_4 as evident by clear photomicrographs and biochemical markers of liver damage which were normal in the rats treated with Liver gen (Plates 1-4).

CONCLUSION

Liver gen® administration had a protective effect against CCl_4-induced acute hepatic damage in rats. The hepatoprotective effect of *Liver gen*® may be linked to its ability to the inhibit lipid peroxidation in the liver and its potential to scavenge free radicals. It however presented with anti hemopoietic characteristic by its reduction of PCV and immunosuppressive action by the reduction of WBC. These are side effects that need to be properly assessed.

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TABLES CAPTION

Table 1: Effects of Liver gen on the Liver marker enzymes in the serum of control and experimental animals

PARAMETERS	Negative Control (Group 1)	CCl_4 + Liver gen® 28.6mg/kg (Group 2)	CCl_4 + Liver gen® 57.2mg/kg (Group 3)	Normal Control (Group 4)
ALP (U/L)	296.5 ± 203.6^{ac}	158.5 ± 46.1^{ac}	223.0 ± 75.1^{ac}	168.5 ± 48.2^{ac}
AST (U/L)	152.6 ± 29.5^{ac}	151.1 ± 15.3^{ac}	132.7 ± 19.96^{ac}	144.2 ± 21.97^{ac}
ALT (U/L)	56.5 ± 15.1^{d}	40.3 ± 7.07^{d}	40.4 ± 6.26^{d}	35.4 ± 6.16^{d}

Values are Mean ±SEM, n=5 in each group. ^{a,c,d}Values in the same row with different superscripts are significantly different at $P<0.05$.

Table 2: Effects of Liver gen on the haematological parameters

PARAMETERS	Negative Control (Group 1)	CCl_4 + Liver gen 28.6mg/kg (Group 2)	CCl_4 + Liver gen 57.2mg/kg (Group 3)	Normal Control (Group 4)
Hb (mg/dl)	11.67 ± 2.22^{ac}	11.40 ± 1.86^{ac}	9.50 ± 2.68^{ac}	11.63 ± 3.38^{ac}
PCV (mg/dl)	39.6 ± 7.18^{ac}	38.9 ± 4.57^{e}	31.64 ± 8.89^{e}	39.4 ± 12.6^{ac}
WBC (mg/dl)	14.3 ± 2.10^{ac}	43.1 ± 32.8^{e}	10.96 ± 4.01^{e}	42.23 ± 28.2^{ac}

Values are Mean ±SEM, n=5 in each group. ^{a,c,e}Values in the same row with different superscripts are significantly different at $P<0.05$.

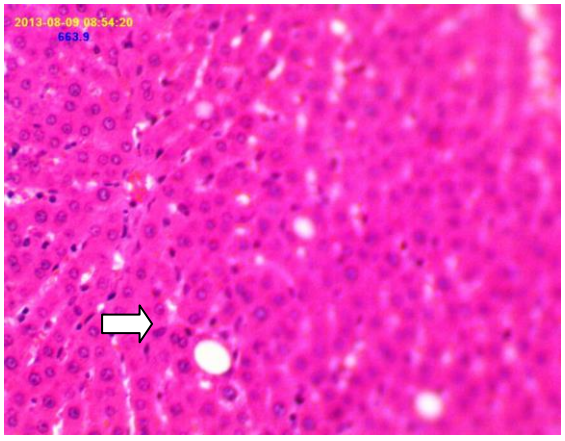
Table 3: Effects of Liver gen on the antioxidant status of liver in the control and experimental animals

PARAMETERS	Negative Control (Group 1)	CCl_4 + Liver gen 28.6mg/kg (Group 2)	CCl_4 + Liver gen 57.2mg/kg (Group 3)	Normal Control (Group 4)
Reduced Glutathione	1.69 ± 0.24^{b}	4.33 ± 0.49^{be}	1.83 ± 0.15^{be}	1.34 ± 0.13^{d}
Lipid Peroxidation	0.05 ± 0.009^{b}	0.39 ± 0.024^{bde}	0.06 ± 0.008^{e}	0.05 ± 0.008^{d}
Glutathione-S-Transferase	0.74 ± 0.12^{b}	2.89 ± 0.36^{bde}	0.99 ± 0.11^{e}	0.75 ± 0.22^{d}
Superoxide dismutase	$2.5e\text{-}5\pm1.3e\text{-}4^{ac}$	$6.1e\text{-}4\pm5.5e\text{-}4^{ac}$	$1.3e\text{-}4\pm6.3e\text{-}6^{ac}$	$3.3e\text{-}4\pm4.3e\text{-}4^{ac}$
Catalase	0.35 ± 0.60^{ac}	1.13 ± 1.96^{ac}	0.04 ± 0.05^{ac}	0.29 ± 0.50^{ac}

Values are Mean ±SEM, n=5 in each group. ^{a,b,c,d,e}Values in the same row with different superscripts are significantly different at $P<0.05$.

FIGURE CAPTION

1



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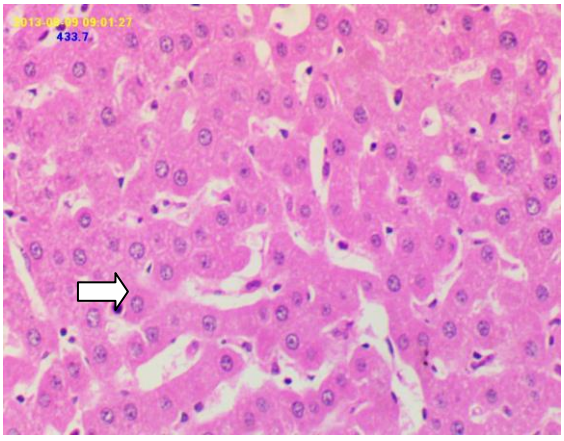

Plates 1-2:Photomicrographs of rat liver treated treated with CCl_4 and CCl_4 + 28.6mg/kg Liver gen® respectively

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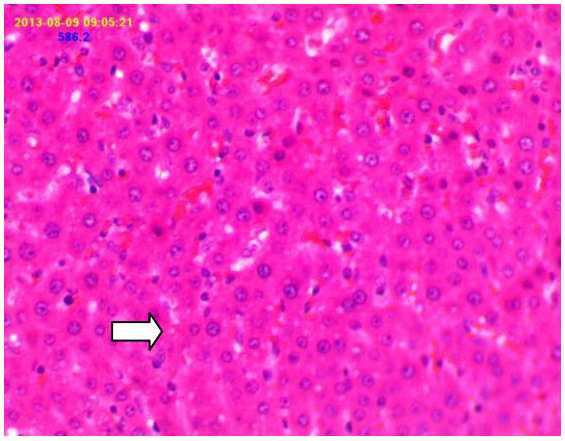
3



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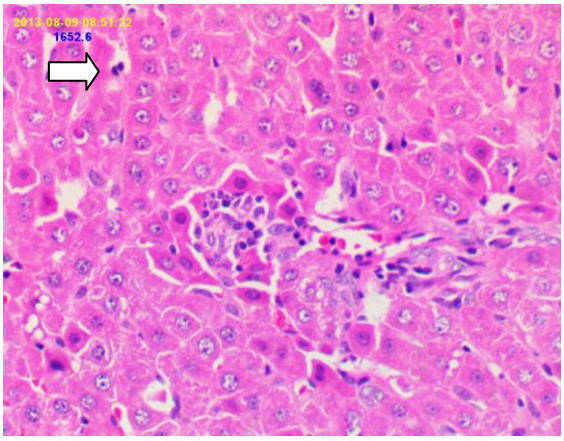

Plates 3-4: Photomicrographs of rat liver treated with CCl_4 and 57.2mg/kg of liver gen Control group respectively

ANTIDIABETIC EFFECTS OF CHITOSAN CAPSULE ON STREPTOZOTOCIN-INDUCED DIABETIC RATS AND POSSIBLE CARDIOPROTECTIVE AND B-CELL REGENERATIVE EFFECTS

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ABSTRACT
Background: Chitosan capsule™ is manufactured by Green World Natural Solutions International Limited, U.S.A. As a food supplement, it is indicated in the management of obesity and hyperglycemia. **Objective:** To investigate the antidiabetic effects of Chitosan capsule in streptozotocin-induced diabetic rats. **Methods:** Twenty male Wistar albino rats (130-150 g) were divided into five groups comprising normal control, diabetic control and diabetic treated with 150 mg/kg body weight chitosan, 300 mg/kg body weight chitosan or 6 IU/kg body weight insulin. Diabetes was induced by a single intraperitonial injection of 65 mg/kg body weight streptozotocin prior to the commencement of treatment. After four weeks of treatment, the animals were sacrificed and their blood samples were collected for biochemical assays. The pancreas was harvested for histological assessments. **Results:** Treatment with the two doses of Chitosan significantly ($p < 0.05$) reduced the blood glucose level, total cholesterol, triglyceride, low density lipoprotein (LDL) cholesterol, and very low density lipoprotein (VLDL) cholesterol but significantly ($p < 0.05$) increased catalase activity compared to the diabetic control. Only the animals treated with the higher dose Chitosan (300 mg/kg body weight) showed a significant ($p < 0.05$) increase in high density lipoprotein (HDL) cholesterol with a significant decrease ($p < 0.05$) in malondialdehyde content, alanine amino transferase (ALT) activity, and alkaline phosphatase (ALP) activity. The aspartate transaminase (AST) activity of the Chitosan treated groups was reduced but not significantly compared to the diabetic control. Histological examination of the pancreas of the diabetic rats treated with the two doses of Chitosan showed increased islet cells cluster compared with the diabetic control group. **Conclusion:** Chitosan capsule posseses hypoglycemic, hypolipidemic and antioxidant potentials, and could therefore be effective in the management of diabetes.

***Keywords**: Chitosan capsule™, Green World, Diabetes, Streptozotocin.*

INTRODUCTION

Diabetes mellitus is a well-known metabolic disease that often leads to many physiological complications, including cardiovascular diseases, renal diseases, and retinal damage [1]. It is divided into two major types, the insulin-dependent (Type 1) and the non- insulin-dependent (Type 2) diabetes mellitus [2]. Although the two types of diabetes have different pathogenic mechanisms, they are both characterized by hyperglycemia, disturbed carbohydrate, protein and fat metabolism and complications of the eyes, kidneys and nerves. Both types constitute a major cause of morbidity and death [3]. The pathologic mechanisms of the disease are mainly from the impaired insulin secretion by the pancreatic β-cells as well as insulin resistance in the target tissues, including the skeletal muscles and the liver, leading to hyperglycemia [4].

Leptin, an adipocyte secreted hormone/cytokine (Adipokine) that regulates appetite and energy metabolism is known to play a major role in islet cell growth and insulin secretion. It is a key anorexigenic signal that maintains normal energy homeostasis [5]. It promotes weight loss, which in turn has a beneficial effect on diabetes control. Impaired insulin action is closely linked

to the phenomenon of obesity. The adipose tissue not only releases free fatty acids but also hormones and cytokines such as leptin, adiponectin, resistin, TNF-alpha and other particles which modify insulin action. Hence the role of obesity in the generation of resistance to insulin and subsequently leading to impaired glucose tolerance as well as overt type 2 diabetes mellitus has been established [6]. Low circulating levels of insulin is mandatory for the antidiabetic actions of insulin [7].

Furthermore, in diabetes mellitus, hyperglycemia generates the reactive oxygen species (ROS), which in turn cause lipid peroxidation and membrane damage, playing an important role in the occurrence of the secondary complications [8]. Therefore, the control of the blood glucose level is an effective strategy for preventing or reversing the diabetic complications and improving the quality of life in both Type 1 and 2 diabetic patients [9]. Recently, the management of diabetic hyperglycemia has attracted much attention in alternative aspect [10]. Some natural products (e.g Chitosan) are thought to improve diabetic hyperglycemia [11]. Thus, study of natural products and supplements in managing diabetic disorders might be beneficial.

Chitosan capsule is one of the supplements produced by Green World Natural Solutions International Limited, USA. It is made up of effective chitosan which comes from natural and pollution-free marine organisms such as shrimp, prawn and crab. Each capsule contains 400mg Chitosan. Chitosan was first discovered in 1859 by Professor C. Rouget [12] and is now incorporated into capsules and is increasingly being used particularly in the United States as an over-the-counter cholesterol-lowering agent. It is the deacetylated form of chitin, an aminopolysaccharide found in the exoskeleton of arthropods and certain fungi [13]. It is called 'the sixth element of life'. It is known to remove free radicals, restrain the absorption of glucose in the blood, decrease the blood lipids, and prevent artery ossification and diabetes. It also has been implicated in the consolidation of immunity and retardation of ageing process [11].

Chitosan marketed as an effective slimming agent with hypoglycemic effects has been evaluated for its anti-obesity effects [14, 15] but its anti-hyperglycemic potential is inconclusive till date, making the study on the effects of Chitosan capsule on the management of diabetic hyperglycemia and some complications of diabetes imperative.

This study was designed to investigate the effects of Chitosan capsule at two different doses (150 and 300 mg/kg body weight) on streptozotocin-induced diabetic rats by examining its effects on body weight, blood glucose, lipid profile, atherogenic indices, liver antioxidants and plasma hepatospecific enzymes.

METHODS

Drugs and Reagents

Chitosan capsuleTM (World (Tianjin) Nutrition & Health Food Co., Ltd., China), Insulin (Novo Nordick A/S, Denmark), Total Cholesterol kit (Biolabo reagents, France), HDLCholesterol kit (Biolabo reagents, France), Triglycerides kit (Biolabo reagents, France), Alkaline Phosphatase kit (Teco Diagnostics, U.S.A.), Aspartate Aminotransferase kit (Randox Laboratories Limited, United Kingdom), Alanine Aminotransferase (Randox Laboratories Limited, United Kingdom), Total Protein kit (Biolabo reagents, France), glacial acetic acid, streptozotocin (Sigma-Aldrich, United Kingdom).

Animal preparation and Housing

Twenty (20) male Wistar albino rats (130-150 g) were purchased from Olu Research Animal Farm, Ibadan, Oyo State, Nigeria. Studies were conducted in compliance with applicable laws and regulations. The rats were weighed and sorted into five groups of four animals each with their average weights approximately equal. The animals were housed in plastic cages at the animal house in the College of Medicine, University of Lagos. All animals were exposed to an environment of 12 hour light: 12 hour dark period, at room temperature. After a one week

acclimatization period on growers mash, the animals were fasted overnight, and their baseline fasting blood glucose level was determined using Accu-Chek Active glucometer and strips, by collecting blood via tail cut. The animals were allowed free access to water and feed and their weights were monitored weekly.

Experimental induction of Diabetes

Sixteen of the animals were rendered diabetic by a single intraperitonial injection of 65mg/kg body weight (bw) of streptozotocin (freshly prepared solution in 0.1 M citrate buffer, pH 4.5) after an overnight fast. Streptozotocin-injected animals exhibited massive hyperglycemia (determined using Accu-Chek Active glucometer and strips). The animals with blood glucose more than 220 mg/dl after 7 days were considered diabetic and used for the experiment [16].

Experimental design

The twenty male Wistar albino rats were divided into five groups (four rats per group); one normal control group (group I) and four diabetic groups (group II, III, IV, and V). All the groups were treated with either Chitosan (dissolved in 1% glacial acetic acid) or 1% glacial acetic acid [16] for four weeks (28 days) as described in Table 1.

Sacrifice of Animals

At the end of the fourth week of treatment, the animals were fasted and sacrificed by cervical dislocation. Blood samples were collected by ocular puncture via heparinzed capillary tubes, into labeled heparinzed bottles. They were centrifuged at 5000 revolution per minute for 5 minutes to obtain the plasma (supernatant). The plasma samples obtained were then analyzed for triglyceride, total cholesterol, HDL cholesterol, alkaline phosphatase, aspartate aminotransferase, alanine transaminase, total protein, catalase, reduced glutathione, superoxide dismutase, malondialdehyde, LDL and VLDL cholesterol, with atherogenic indices also calculated. The pancreas and liver of all animals were harvested, weighed and preserved in 10 % formaldehyde (formalin) solution for histological studies.

Biochemical Assay

Determination of Fasting Blood Glucose Level

The fasting blood glucose concentration was determined weekly using the Accu-Chek Active glucometer and strips (The glucose contained in the sample reacts with the glucose oxidase enzyme in the glucose electrode strips to produce an electric current. The magnitude of the current produced by the electrodes is directly proportional to the glucose concentration).

Lipid Profile Assay

Plasma triglyceride was assayed enzymatically with Biolabo commercial test kits (Biolabo reagents, France), total cholesterol concentrations were assayed enzymatically with Biolabo commercial test kits (Biolabo reagents, France) while high density lipoprotein was isolated with Biolabo commercial test kits from Biolabo reagents, France [17, 18, 19]. Cholesterol concentration of the high density lipoprotein (HDL cholesterol) was determined, as in total cholesterol. Plasma very low density lipoprotein (VLDL) and low density lipoprotein (LDL) cholesterol concentrations were calculated using the Friedewald equation [20]. The atherogenic indices were calculated as reported by prevoious workers [21, 22].

Liver Antioxidants Assay

The tissue (liver) of the rats were washed, 0.5g of the tissue was crushed and homogenized in 4.5ml of phosphate buffer solution. The homogenate was centrifuged. The supernatant was decanted and examined for the various antioxidants. The activities of catalase and superoxide

dismutase were determined as previously described [23, 24] along with glutathione and malondialdehyde contents [25, 26] respectively.

Hepatospecific Enzymes Assay

The plasma activities of alanine transaminase (ALT) and aspartate transaminase (AST) were determined using Randox test kits, Randox Laboratories Limited, United Kingdom [27] while the plasma activities of alkaline phosphatase (ALP) were determined using Teco test kits (Teco Diagnostics, U.S.A.).

Histological Study of the Pancreas

The tissues were fixed in 10% formalin solution for ten days. They were then dehydrated through ascending grades of alcohol to absolute alcohol (50%, 70%, 90%, 95%, absolute I, II and III alcohol for 30 minutes each respectively). The tissues were left in xylene overnight. Wax was kept in an electrically heated and thermometrically controlled oven maintained at about 56-57^{0}C to maintain it in molten form. The tissues were then transferred into the molten wax for 30 minutes for impregnation. After this, the tissues were embedded in solidified wax. 5µm thick sections were cut using a microtome and stained with haematoxylin and eosin [28]. The specimens were evaluated with light microscope. All histopathological changes were examined by pathologist.

Statistical Analysis

Data were presented as the mean ± standard error of the mean (S.E.M) and n represents the number of rats per group. All statistical comparisons were made with one-way analysis of variance (ANOVA) followed by Turkey Multiple Comparison Test using Graph Pad Prism 4.0. A value of $p< 0.05$ indicates significant differences in all cases.

RESULTS

Effects of Chitosan Capsule on Body Weight Changes

Figure 1 shows the effects of Chitosan Capsule (150 and 300mg/kg body weight) on the body weight of streptozotocin induced diabetic rats after 4 weeks of treatment. Induction of diabetes using streptozotocin caused a severe weight loss in the rats. Treatment with chitosan (300 mg/kg body weight) and insulin (6 IU) controlled the body weight loss in diabetic animals, although chitosan did not completely normalize the body weight.

Effects of Chitosan Capsule on Fasting Blood Glucose

Figure 2 shows the fasting blood glucose concentration of the experimental rats after diabetes induction with streptozotocin, while Figure 3 shows the effects of Chitosan Capsule (150 and 300mg/kg body weight) treatment on the fasting blood glucose concentration of streptozotocin induced diabetic rats. The fasting blood glucose concentrations of the streptozotocin diabetic rats were significantly ($p < 0.05$) higher than the normal group. Diabetic rats treated with chitosan showed a dose-dependent response. Treatment with 300 mg and 150 mg/kg bodyweight chitosan significantly ($p< 0.05$) reduced the blood glucose after the third and fourth week of treatment respectively as compared to the diabetic control group.

Effects of Chitosan Capsule on Lipid Profile and Atherogenic Indices

Tables 2 and 3 show the effects of Chitosan Capsule (150 and 300mg/kg body weight) on the lipid profile and atherogenic indices of streptozotocin induced diabetic rats after 4 weeks of treatment. A significant ($p< 0.05$) increase in triglyceride, total cholesterol, VLDL and LDL cholesterol with a decrease in HDL cholesterol was observed in the diabetic rats when compared with the normal rats. Triglyceride, total cholesterol, VLDL and LDL cholesterol were reduced

significantly ($p< 0.05$) in all the treated groups as compared to the diabetic control group with a corresponding increase in HDL cholesterol. Although the increase was not significant in the groups treated with lower dose chitosan and insulin, a higher dose of chitosan significantly ($p< 0.05$) increased the HDL cholesterol as compared to the diabetic control group. A significant ($p< 0.05$) increase in atherogenic indices was observed in diabetic rats when compared with the level in the normal rats. Treatments for 4 weeks caused a significant ($p< 0.05$) reduction in the atherogenic indices in all treated groups as compared to the diabetic control group.

Effects of Chitosan Capsule on Liver Antioxidants

Table 4 shows the effects of Chitosan Capsule (150 and 300mg/kg body weight) on the liver antioxidants of streptozotocin induced diabetic rats after 4 weeks treatment. Although not statistically significant, the reduced glutathione content of the treated groups was increased when compared to the diabetic control. A significant ($p< 0.05$) increase in catalase activity was observed in diabetic rats when compared with the level in the normal rats. Catalase activity was significantly increased in all the treated groups while only the groups treated with Chitosan Capsule (300mg/kg body weight) and Insulin showed significant ($p< 0.05$) decrease in malondialdehyde content as compared to the diabetic control. No significant change in superoxide dismutase activity was observed in all the groups.

Effects of Chitosan Capsule on Plasma Hepatospecific Enzymes

Table 5 shows the effects of Chitosan Capsule (150 and 300mg/kg body weight) on the plasma hepatospecific markers of streptozotocin induced diabetic rats after 4 weeks of treatment. A significant ($p< 0.05$) increase in AST, ALT and ALP was observed in diabetic rats when compared with the level in the normal rats. ALP activity was significantly ($p< 0.05$) decreased in all the treated groups while only the higher dose Chitosan and Insulin treatment significantly reduced the ALT activity. Treatment with Chitosan reduced AST activity, but only the Insulin treated group showed a significant decrease.

Effects of Chitosan Capsule on the Histology of the Pancreas

Figure 4 shows the effects of Chitosan Capsule (150 and 300mg/kg body weight) on the histology of the pancreas of streptozotocin induced diabetic rats after 4 weeks of treatment. The diabetic control group has reduced islet cell clusters as compared to the normal group (non-diabetic). Although not as much as the normal group, the treated groups have increased islet cell clusters as compared to the diabetic control group.

DISCUSSION

In this study, we examined the effects of two different doses of chitosan capsule (150 and 300 mg/kg body weight) on the management of streptozotocin induced diabetes. The study was designed to determine whether or not therapeutic intervention with this capsule at the two doses would help in the management of diabetes.

Streptozotocin-induced diabetes is characterized by severe weight loss [29] which was also observed in this study. The decrease in body weight of the diabetic rats might be due to muscle wasting caused by unavailability of carbohydrate as an energy source [30]. Treatment with the higher dose chitosan and insulin controlled the body weight loss in diabetic animals although the chitosan did not completely normalize the body weight. This body weight control might be due to improvement in the glucose metabolism of the treated rats.

The study has shown that administration of streptozotocin caused a significant increase in the blood glucose level (hyperglycemia) of the treated rats throughout the study period compared to the normal group. The fundamental mechanism behind this involves over production (excessive hepatic glycogenolysis and gluconeogenesis) and decreased utilization of glucose by the tissues

[16]. Diabetic animals treated with chitosan showed a dose-dependent response. The hypoglycemic effect observed is consistent with the histological examination of the pancreas after four weeks of treatment in which the treated groups have increased islet cell clusters as compared to the diabetic animals which have reduced islet cell clusters. The blood glucose-lowering action of chitosan may be due to its triglyceride-lowering action and β-cell regeneration [16].

Previous studies indicate that decrease in plasma high density lipoprotein (HDL) cholesterol otherwise known as 'good cholesterol' and elevated levels of plasma very low density lipoprotein (VLDL) and low density lipoprotein (LDL) are risk factors for cardiovascular disease [31, 32]. Chitosan treatment significantly increased the plasma HDL cholesterol and decreased the levels of plasma VLDL and LDL with the higher dose treatment showing the greater effect. The result obtained from this study therefore indicates a cardioprotective effect of chitosan at the two doses. The mechanism by which chitosan performs this function might be due to its high positive charge density that enables it to interact with negative surfaces such as lipids [16]. Atherogenic indices are also good markers of accessing the risk of developing cardiovascular disease [21]. Significant reduction in the atherogenic indices of all treated groups as compared to control also confirms the cardioprotective power of chitosan which is more pronounced in the higher dose chitosan treated group.

Streptozotocin-induced diabetes is also characterized with an increase in thiobarbituric acid reactive substances such as malondialdehyde, an indirect evidence of intensified free radical production. Malondialdehyde is a very reliable index of oxidative stress and lipid peroxidation [33, 34]. Progressive glycation of the enzymatic proteins accounts for the reduced activities of the antioxidant enzymes in the diabetics [33, 35, 36]. The significant reduction in the malondialdehyde content and the improved catalase activity of the chitosan treated diabetic rats indicate its protective effect against oxidative stress in the liver.

The result obtained from this study indicates significant increase in the plasma activities of AST, ALT and ALP of the streptozotocin-diabetic rats. Previous studies have shown that the elevation of plasma hepatospecific markers; AST, ALT and ALP reflect the damage of hepatic cells [37, 38] which may be caused by streptozotocin. It has been postulated that diabetes could induce defects in sarcolemmal enzymatic activities which also lead to such effects [39]. However, it was observed in this study that chitosan treatment decreased the plasma activities of AST, ALT and ALP of the streptozotocin-diabetic rats with the higher dose chitosan treatment showing a greater effect indicating its protective effect over the liver and improvement in liver function efficiency.

In conclusion, the findings in this present study indicate the hypoglycemic, hypolipidemic and antioxidant effects of Chitosan capsule, thereby revealing its cardioprotective and β–cell regenerative potentials in streptozotocin diabetic rats.

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Table 1: Experimental Design

S/N	Group	Treatment
1	Normal	1% glacial acetic acid (2ml/ kg bodyweight)
2	Diabetic control	1% glacial acetic acid (2ml/ kg bodyweight)
3	Diabetic + 150 mg/kg bw Chitosan	150 mg Chitosan/kg body weight in 1% glacial acetic acid
4	Diabetic + 300 mg/kg bw Chitosan	300 mg Chitosan/kg body weight in 1% glacial acetic acid
5	Diabetic + Insulin (6IU/kg bw)	6 IU Insulin/kg body weight in 1% glacial acetic acid

The fasting blood glucose level was measured weekly among all groups until sacrifice using Accu-Check Active glucometer and strips by collecting blood via tail cut.

Table 2: The effects of Chitosan Capsule (150 and 300mg/kg body weight) on lipid profile of streptozotocin induced diabetic rats after 4 weeks treatment.

Treatment Group	Concentration (mmol/L)				
	Total Cholesterol	Triglyceride	HDL Cholesterol	VLDL Cholesterol	LDL Cholesterol
Normal	1.78±0.14	0.46±0.03	0.93±0.08	0.21±0.02	0.64±0.08
Diabetic Control	3.20±0.21*	2.42±0.65*	0.70±0.02*	1.10±0.30*	1.39±0.38*
Diabetic + 150mg/kg bw Chitosan	1.83±0.21[a]	0.83±0.14[a]	1.16±0.21	0.38±0.06[a]	0.29±0.11[a]
Diabetic + 300mg/kg bw Chitosan	1.78±0.12[a]	0.42±0.01[a]	1.43±0.05[a]	0.19±0.00[a]	0.16±0.08[a]
Diabetic + Insulin (6IU/kg bw)	1.86±0.29[a]	0.55±0.01[a]	1.08±0.19	0.25±0.00[a]	0.53±0.14[a]

Values are Mean ±SEM, n=4 in each group. *Significant change in comparison with normal group at $p < 0.05$. [a]Significant change in comparison with diabetic control group at $p < 0.05$.

Table 3: The effects of Chitosan Capsule (150 and 300mg/kg body weight) on atherogenicindices of streptozotocin induced diabetic rats after 4 weeks treatment.

Treatment group	Cardiac Risk Ratio	Atherogenic Coefficient	Atherogenic Index of Plasma
Normal	1.92±0.09	0.92±0.09	-0.31±0.01
Diabetic Control	4.57±0.31*	3.57±0.31*	0.47±0.17*
Diabetic + 150mg/kg bw Chitosan	1.72±0.33[a]	0.72±0.33[a]	-0.14±0.13[a]
Diabetic + 300mg/kg bw Chitosan	1.24±0.04[a]	0.24±0.04[a]	-0.54±0.22[a]
Diabetic + Insulin (6IU/kg bw)	1.78±0.19[a]	0.78±0.19[a]	-0.28±0.07[a]

Values are Mean ±SEM, n=4 in each group. *Significant change in comparison with normal group at $p < 0.05$. [a]Significant change in comparison with diabetic control group at $p < 0.05$.

Table 4: The effects of Chitosan Capsule (150 and 300mg/kg body weight) on the liver antioxidants of streptozotocin induced diabetic rats after 4 weeks treatment.

Treatment group	Reduced Glutathione content (µmol/ml)	Catalase activity (µmol/ml)	Malondialdehyde content ($x10^{-6}$ µmol/ml)	Superoxide dismutase activity ($x10^{-3}$ µmol/ml)
Normal	5848.00±273.4	11.07±0.93	6.58±1.71	7.87±0.18
Diabetic Control	5447.00±462.5	6.332±1.074*	8.42±1.67*	7.97±0.41
Diabetic + 150mg/kg bw Chitosan	6162.00±150.8	12.32±0.67a	6.90±1.08	8.61±0.52
Diabetic + 300mg/kg bw Chitosan	6390.00±256.2	22.63±0.40a	3.96±.1.01a	8.79±0.14
Diabetic + Insulin (6IU/kg bw)	5787.00±337.4	13.28±0.06a	1.89±0.08a	8.04±0.12

Values are Mean ±SEM, n=4 in each group. *Significant change in comparison with normal group at $p < 0.05$. [a]Significant change in comparison with diabetic control group at $p < 0.05$.

Table 5: The effects of Chitosan Capsule (150 and 300mg/kg body weight) on the plasma hepatospecific markers of streptozotocin induced diabetic rats after 4 weeks treatment.

Treatment group	Aspartate transaminase (U/L)	Alanine transaminase (U/L)	Alkaline phosphatase (U/L)
Normal	14.00±3.89	13.25±3.43	18.25±0.48
Diabetic Control	50.50±9.54*	40.50±5.87*	24.25±1.03*
Diabetic + 150mg/kg bw Chitosan	34.00±8.38	28.75±7.97	19.25±0.48a
Diabetic + 300mg/kg bw Chitosan	32.00±9.65	18.75±4.31a	18.75±.0.48a
Diabetic + Insulin (6IU/kg bw)	31.50±1.04a	15.25±3.61a	20.75±2.50a

Values are Mean ±SEM, n=4 in each group. *Significant change in comparison with normal group at $p < 0.05$. [a]Significant change in comparison with diabetic control group at $p < 0.05$.

FIGURE CAPTION

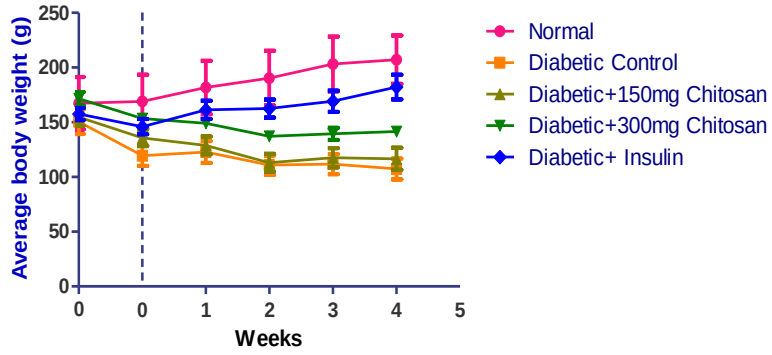

Figure 1: The effects of Chitosan Capsule (150 and 300mg/kg body weight) on the body weight of streptozotocin induced diabetic rats. Values are Mean ±SEM, n=4 in each group.

Legend:

0= Average body weight 7 days after diabetes induction (before treatment)
1= Average body weight after 1 week treatment
2= Average body weight after 2 weeks treatment
3= Average body weight after 3 weeks treatment
4= Average body weight after 4 weeks treatment

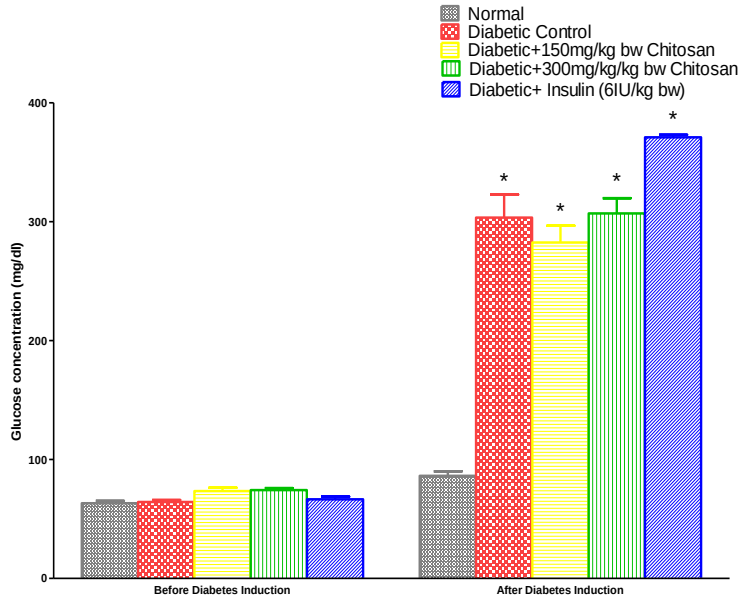

Figure 2: Fasting blood glucose concentration (mg/dl) of rats before and after diabetes induction using streptozotocin. Values are Mean ±SEM, n=4 in each group. *Significant change in comparison with normal group at $p < 0.05$.

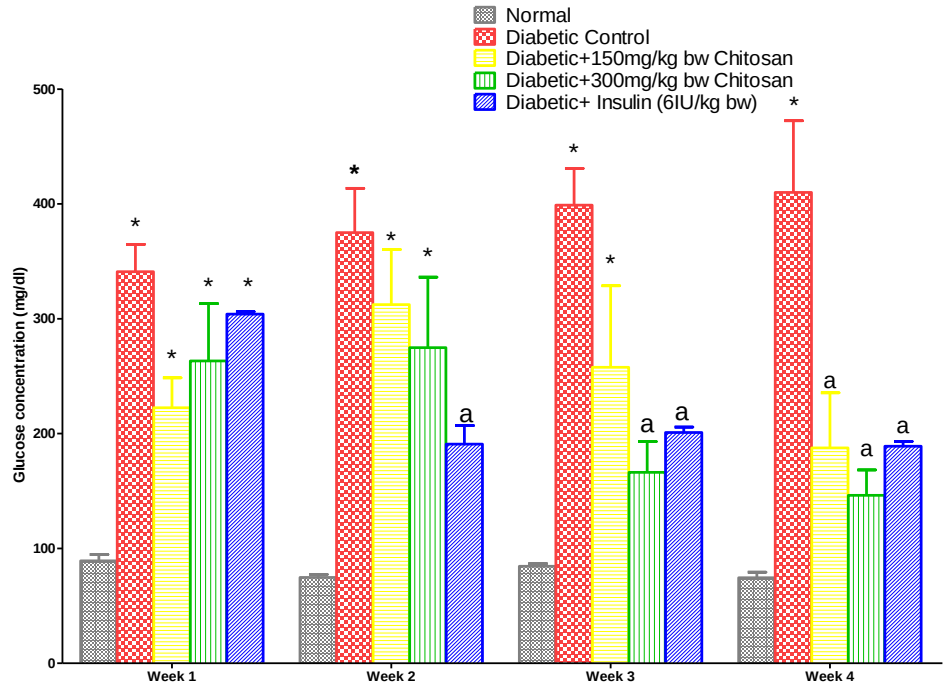

Figure 3: The effects of Chitosan Capsule (150 and 300mg/kg body weight) on the fasting blood glucose concentration of streptozotocin induced diabetic rats after 1 week, 2 weeks, 3 weeks and 4 weeks treatment. Values are Mean ±SEM, n=4 in each group. *Significant change in comparison with normal group at $p < 0.05$. [a]Significant change in comparison with diabetic control group at $p < 0.05$.

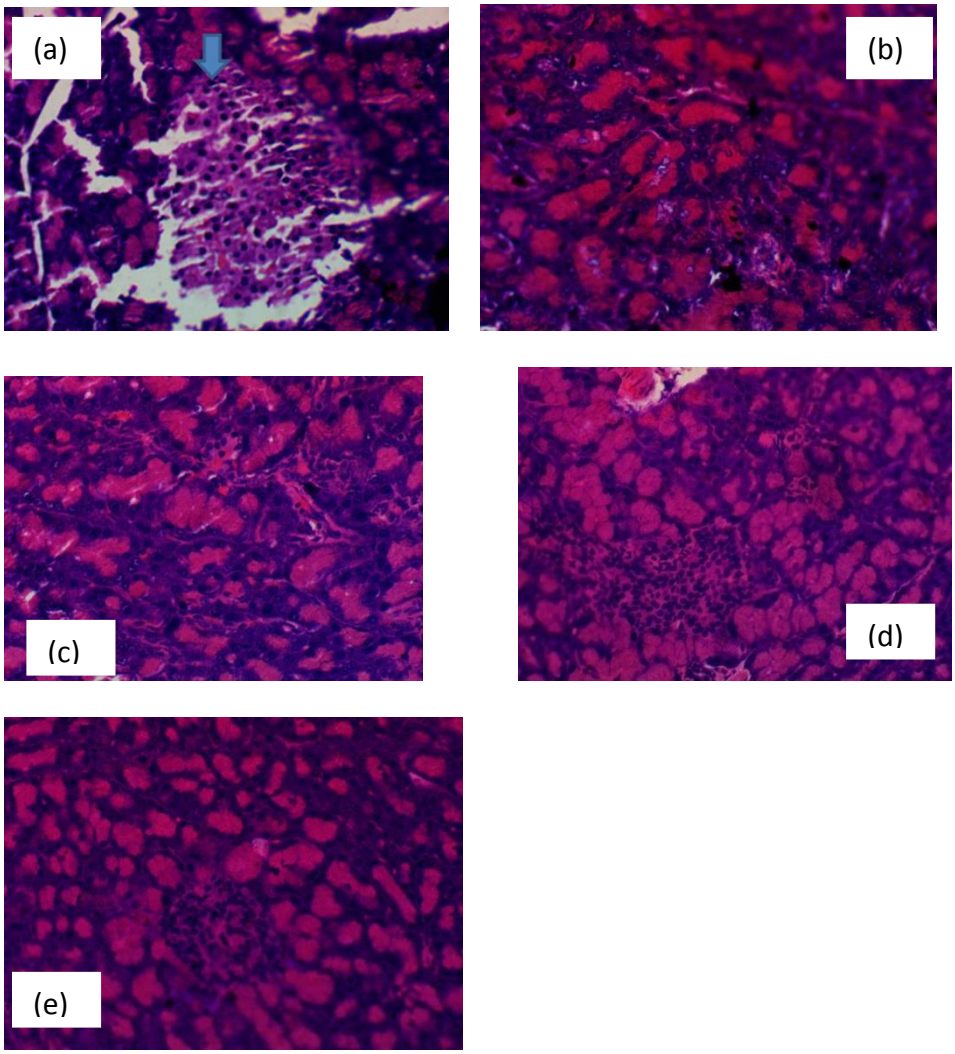

Figure 4: Histological photograph showing the pancreas of (a) normal rat. Arrow shows islet cells cluster, (b) streptozotocin-diabetic rat (diabetic control). Paucity of islet cells was observed, (c) streptozotocin-diabetic rat treated with 150 mg/kg body weight Chitosan for four weeks (diabetic + 150 mg Chitosan). Islet cells cluster was seen, (d) streptozotocin-diabetic rat treated with 300 mg/kg body weight Chitosan for four weeks (diabetic + 300 mg Chitosan). Islet cells cluster was seen, (e) streptozotocin-diabetic rat treated with 6IU/kg body weight Insulin for four weeks (diabetic + Insulin). Islet cells cluster was seen.

ESTROUS CYCLE STUDY IN GREEN COCONUT (COCOS NUCIFERA) WATER TREATED PSEUDOPREGNANT FEMALE SPRAGUE-DAWLEY RATS

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ABSTRACT
Background: Pseudopregnancy is a serious endocrine, emotional and psychological cause of female infertility. **Objective:** This study investigated the effect of green coconut water on estrous cycling pattern in pseudopregnant female Sprague-Dawley rats. **Material and Methods:** Sixty- five cyclic female Sprague-Dawley rats (145-170 g) were used for this study. The animals were divided into four experimental study groups, Group A were made pseudo-pregnant and treated with 5 and 10 ml/100g body weight of green coconut water (GCW) for 4, 8 and 10 days respectively. Group B (pseudo-pregnancy control rats), Group C (pregnancy control rats) and Group D (control rats) received normal saline for 4, 8 and 10 days. **Result:** There were no cycles completed in the pseudopregnant treated and pseudopregnant and pregnancy control groups. **Conclusion:** The administration of GCW did not shorten the lengths of pseudo-pregnancies in female rats.

Keywords: *Estrous cycle, Green coconut water, Pseudopregnancy*

INTRODUCTION

Pseudo-pregnancy also known as false, imaginary, simulated, phantom or hysterical pregnancy is a condition when symptoms of pregnancy are experienced in the absence of actual pregnancy. During pseudopregnant state, symptoms such as, weight gain, nausea, amenorrhea, enlarged abdomen, morning sickness, breast tenderness and imagined fetal movements are experienced. Women sometimes test positive on a pregnancy test and about one percent of women eventually experience false labor (1).

This phenomenon is referred to as pseudocyesis in human. It is rare in developed countries while common in developing countries where illiteracy, poverty, poor medical facilities (ultrasonographic machines), disturbed family dynamics, societal pressure and belief about generation continuity are major concerns (2, 3, 4). It is generally estimated that false pregnancy is caused by changes in the endocrine system which translate the corpus luteum of menstruation to corpus luteum of pregnancy with physical changes similar to those of pregnancy (5, 6, 7).

The green coconut water is the juice of an immature coconut fruit. It is a valuable source of medicine that literally comes in its own container. It is use to conquer irregular or painful menstruation. Also taken during pregnancy to give the unborn babies strength and vitality and also aid the maintain of pregnancy, hence use in women with threatened or habitual abortion (8, 9). Women and men in their mid-60s, have reported to have increased libido after drinking the coconut water. It also improves sexual vitality, boosts sperm count and enhances motility (10).

METHODS

Immature coconut fruits

The immature coconut fruits were purchased from William kudofoke coconut farm in Ajara - topa, Badagry, Lagos. The average weight of the fruit was 1.55 kg. The fruit was authenticated in the forest herbarium, Ibadan with ascension number FHI 109665.

Extraction of the green coconut water
The unripe coconut fruits were washed and dehusked. The extraction of the water was done through the germinal pore, poured directly into an airtight bottle and kept refrigerated. The GCW was replaced every three weeks (11, 12).

Experimental Animals
A total of Sixty-five adult female SD- rats weighing 145-170 g were obtained from the Nigerian Institute of Medical Research, Yaba, Lagos and were authenticated in the department of Zoology of the University of Lagos. The animals were kept in standard plastic cages in the animal house of the Department of Anatomy and allowed to acclimatize for two weeks under standard laboratory conditions of room temperature 27ºC with a photoperiodicity of twelve hours light alternating with twelve hours of darkness. The animals had free access to clean tap water and pellets.

Establishment of estrous cyclicity
The rats went through a recruitment phase of establishing estrous cyclicity, determined from the cytology of their vaginal smears obtained daily between 8.00a.m and 10.00 a.m. Normal saline was drawn into the tip of the pipette and inserted 2mm deep into the vaginal canal and 2 drops emptied into the vaginal cavity. The mixture of vaginal fluid and normal saline was suctioned back into the tip of the pipette. The smear was placed on glass slide and examined under the light microscope using the 40X objective lens immediately before drying up (13, 14).

Induction of pseudo-pregnancy
Vagino-cervical stimulation provided by manual probing with thin iron rod was used to induce false pregnancy. This process was carried out on the estrus day (heat period) of the cycle (15).

Treatment protocol
The animals with established estrous cyclicity of 4 days were randomly divided into four (4) major experimental groups I to VI. GCW at 5 and 10 ml/100gbw daily in two divided doses were administered orally to reestablish estrous acyclicity in hyperprolactine rats (16).
Group I animals were made pseudopregnant and subdivided into 6 subgroups (Ia, b, c, d, e, and f) of 5 rats each treated with 5 ml/100g and 10 ml/100gbody weight/day through oral route in 1a & d, 1b & e and 1c & f respectively for 4, 8 and 10days. In group II, the rats were made pseudopregnant and subdivided into 3 subgroups (Ia, b and c) of 5 rats each, received distilled water only for 4, 8 and 10days respectively. The group III rats were truly pregnant subdivided into 3 subgroups (Ia, b and c) of 5 rats each, received distilled water only for 4, 8 and 10 days respectively. The group VI rats were the control group. All procedures involving animals in this study conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding Principles in the Care and Use of Animals.

Total body weight gain evaluation
The body weights were measured with a weighing balance. The differences in the weight were estimated as the weight gain and expressed as g/100 g body weight of the corresponding weight.

Statistical analysis
Results were expressed as means ± standard deviation (SD) and subjected to statistical analysis using one-way analysis of variance (ANOVA) and the Scheffe's post-hoc test. The significance level considered was $p < 0.05$.

RESULTS AND DISCUSSION

The control animals completed a cycle every 4 days with estrous phases changing daily. There were no cycles completed in the pseudopregnant-GCW treated and pseudopregnant and pregnancy control groups as the animals spent more days on the diestrous phase of estrous cycle. There were no reversals of estrous cyclicty in the pseudopregnant rats.

Pseudocyesis has been reported to exhibit Psychodynamic-endocrine control on the gonads. Most of the currently accepted causal theories emphasize an interaction between psychologic factors and the reproductive system with the mediation of hormonal influences (17). Most pseudocyetic women suffer from major depression, anxiety and/or emotional stress due to psychologic conflicts and fear becoming pregnant (18). Depression has deficits in brain dopamine and norepinephrine activities and increased sympathetic nervous system activity (19, 20, 21). And a dysfunction of central nervous system catecholaminergic pathways involved in the regulation of anterior pituitary hormone secretion by decreasing steroid feedback inhibition of gonadotropin releasing hormone (22, 23, 24). Hence the underlying cause of pseudo-pregnancy are complicated and caused by multidimensional factors caused by the impairment of different reproductive hormones mimicking the hormonal changes of true pregnancy.

The decrease in body weight gain in GCW treated groups conforms to the report that the administration of immature coconut water demonstrated significant decrease in total body weight in mice (25).

The Patterns Of Estrous Cycles In Sprague-Dawley Rats

The rats in the Pseudopregnant GCW -treated groups remain in the diestrous phase of the cycle which was comparable with the pseudopregnant and pregnancy control groups.

Body Weight Gain

The Pseudopregnant GCW -treated groups for 8 and 10 days demonstrated significant decreases in weight gain when compared with the pseudopregnant and pregnancy control groups.

CONCLUSION

The findings from this study indicate that GCW did not reverse estrous acyclicty in pseudo-pregnant female rats. Pseudo-pregnancy has been reported to occur as a result of psychological induced reproductive hormonal distruption. However, since the study was carried within short duration because of the short length of pseudo-pregnancy in rat, further studies should be carried out in animals with longer length of pseudo-pregnancy.

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Table 1: Vaginal smear histological representation of each phase of estrous cycle

DAY	PHASES	VAGINAL SMEAR CYTOLOGY
1	Metestrus	leukocytes amidst remnants of large squamous cells
2	Diestrus	predominance of leukocytes and a few large nucleated cells
3	Proestrus	large nucleated cells and few leukocytes
4	Estrous	large flakes of squamous cells

Numbers of Completed Estrous Cycles

Table 2: The Numbers of Completed Estrous Cycles in the Experimental and Control Groups.

GROUPS	SUB-GROUP DETAIL	NUMBER OF ESTROUS CYCLE
I	$GCW_{M/d4days}$	0.00 ± 0.00
	$GCW_{H/d4days}$	0.00 ± 0.00
II	$DSTL_{4days}$	0.00 ± 0.00
III	$DSTL_{4days}$	0.00 ± 0.00
Control	$DSTL_{4days}$	1.00 ± 0.00
I	$GCW_{M/d8days}$	0.00 ± 0.00
	$GCW_{H/d8days}$	0.00 ± 0.00
II	$DSTL_{8days}$	0.00 ± 0.00
III	$DSTL_{8days}$	0.00 ± 0.00
Control	$DSTL_{8days}$	2.00 ± 0.00
I	$GCW_{M/d10days}$	0.00 ± 0.00
	$GCW_{H/d10days}$	0.00 ± 0.00
II	$DSTL_{10days}$	0.00 ± 0.00
III	$DSTL_{10days}$	0.00 ± 0.00
Control	$DSTL_{10days}$	3.09 ± 0.82

All values are expressed as mean ± standard deviation

Key for table 2

I: Pseudo-pregnant treated groups; II: Pseudo-pregnant none-treated group; III: True Pregnancy group; DSTL: Distilled water; $GCW_{M/d}$: 5 ml/100gbw of green coconut water; $GCW_{H/d:}$ 10 ml/100gbw of green coconut water.

Table 3: The Numbers of Days of Phases of Estrous Cycles in the Experimental and Control Groups

GROUPS	SUB-GROUP DETAIL	METAESTROUS	DIESTROUS	PROESTROUS	ESTROUS
I	$GCW_{M/d4days}$	0.00 ± 0.00	4.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	$GCW_{H/d4days}$	0.40 ± 0.55	3.00 ± 0.00	0.00 ± 0.00	0.60 ± 0.55
II	$DSTL_{4days}$	0.80 ± 0.45	3.20 ± 0.45	0.00 ± 0.00	0.00 ± 0.00
III	$DSTL_{4days}$	0.40 ± 0.55	3.20 ± 1.09	0.00 ± 0.00	0.20 ± 0.45
Control	$DSTL_{4days}$	0.98 ± 0.55	1.40 ± 0.09	1.00 ± 0.98	1.20 ± 0.44
I	$GCW_{M/d8days}$	0.40 ± 0.55	7.20 ± 1.30	0.20 ± 0.45	0.40± 0.55
	$GCW_{Hd8days}$	0.40 ± 0.55	7.40 ± 0.55	0.00± 0.00	0.20± 0.45
II	$DSTL_{8days}$	0.40 ± 0.55	7.60 ± 0.55	0.00± 0.00	0.00± 0.00
III	$DSTL_{8days}$	0.60 ± 0.55	7.00 ± 1.00	0.00± 0.00	0.40± 0.55
Control	$DSTL_{8days}$	1.84 ± 0.55	2.09 ± 0.31	1.90 ± 0.47	1.96 ± 0.2
I	$GCW_{M/d10\ days}$	0.40 ± 0.55	8.80 ± 1.64	0.40 ± 0.55	0.40 ± 0.55
	$GCW_{H/d10\ days}$	0.80± 0.45	8.00 ± 1.50	0.60 ± 0.55	0.60± 0.55
II	$DSTL_{10days}$	0.60± 0.55	9.40 ± 0.55	0.00 ± 0.00	0.00 ± 0.00
III	$DSTL_{10days}$	0.80± 0.55	8.80 ± 0.84	0.00 ± 0.00	0.40± 0.55
Control	$DSTL_{10days}$	2.50 ± 0.44	2.99 ± 0.11	2.90 ± 0.05	2.93 ±0.21

All values are expressed as mean ± standard deviation

Key for table 3

I: Pseudo-pregnant treated groups; II: Pseudo-pregnant none-treated group; III: True Pregnancy group; **DSTL**: Distilled water; **$GCW_{M/d}$**: 5 ml/100gbw of green coconut water; **$GCW_{H/d}$**: 10 ml/100gbw of green coconut water

Table 4: The Body Weight Gain In The Experimental And Control Sprague-Dawley Rats

GROUPS	SUB-GROUP DETAIL	BODY WEIGHT GAIN (g/100gbw)
I	$GCW_{M/d\ 4days}$	2.15 ± 0.21
	$GCW_{H/d4days}$	2.17 ± 0.15
II	$DSTL_{4days}$	2.08 ± 0.17
III	$DSTL_{4days}$	2.10 ± 0.00
Control	$DSTL_{4days}$	2.00 ± 0.10
I	$GCW_{M/d8days}$	1.37 ± 0.38 *
	$GCW_{H/d8days}$	1.10 ± 0.10*
II	$DSTL_{8days}$	2.10 ± 0.17
III	$DSTL_{8days}$	2.17 ± 0.15
Control	$DSTL_{8days}$	2.00 ± 0.10
I	$GCW_{M/d\ 10days}$	1.17± 0 .15*
	$GCW_{H/d\ 10days}$	1.01 ± 0.10*
II	$DSTL_{10days}$	2.00 ±0.17
III	$DSTL_{10days}$	2.18± 0.15
Control	$DSTL_{10days}$	2.00 ± 0.10

All values are expressed as mean ± standard deviation

*Significant differences; $p < 0.05$

Key for table 4

I: Pseudo-pregnant treated groups; II: Pseudo-pregnant none-treated group; III: True Pregnancy group; **DSTL**: Distilled water; **$GCW_{M/d}$**: 5 ml/100gbw of green coconut water;
$GCW_{H/d}$: 10 ml/100gbw of green coconut water.

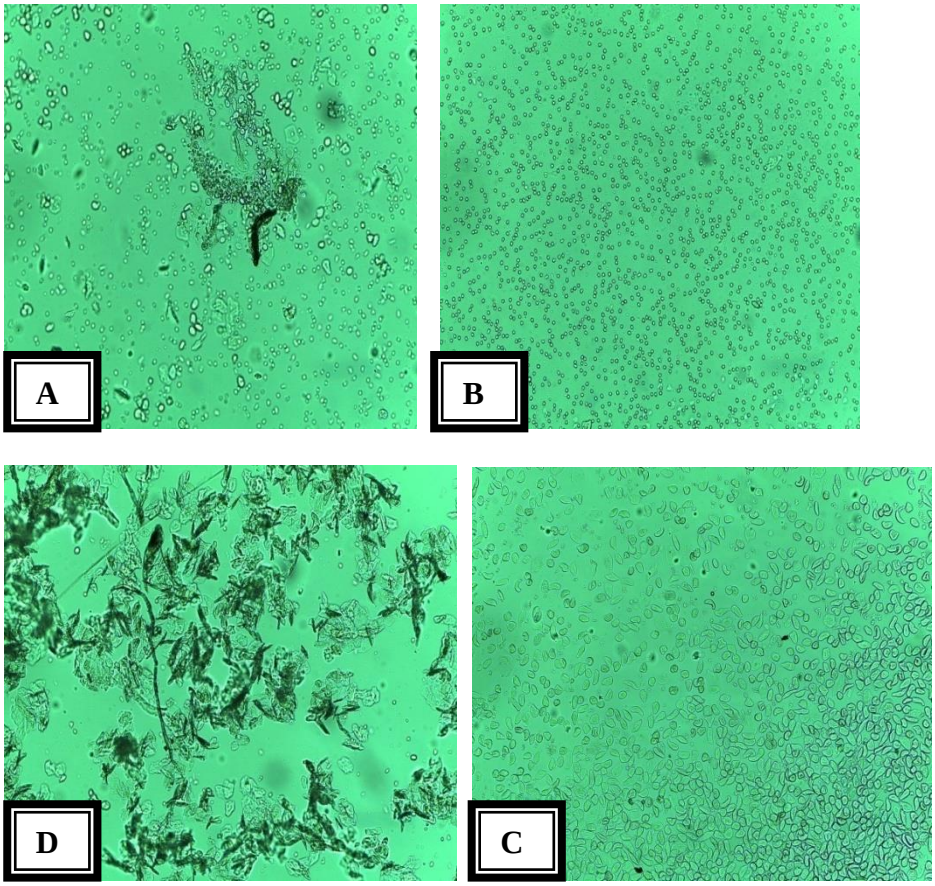

FIGURE 1: Photomicrograph showing the histological evaluations of vaginal smear of each phase of estrous cycle of an individual rat.
A: Metestrus phase; **B**: Diestrus phase; **C**: Proestrus phase; D: Estrus phase.

NICOTINAMIDE AMELIORATES SERUM C-PEPTIDE AND BRAIN TRYPTOPHAN LEVELS IN STZ-INDUCED DIABETIC SPRAGUE- DAWLEY RATS

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ABSTRACT
Metabolic complications of Type-1 diabetes mellitus are a significant source of morbidity and mortality. Nicotinamide treatment has been reported to reverse pancreatic beta cell damage and used to prevent Type-1 diabetes mellitus. The effects of nicotinamide on fasting blood glucose, serum C-peptide and brain tryptophan have not been fully investigated. The objective of the study is to evaluate the effect of nicotinamide on fasting blood glucose, serum C-peptide and brain tryptophan levels in Streptozotocin (STZ)-induced diabetic rats. Type-1 diabetes was induced by intra-peritoneal injection of streptozotocin (55mg/kg) using Sprague-Dawley rats. After 2 days, the rats were divided into diabetic control and nicotinamide-treatment group. Nicotinamide was orally administered at daily doses of 375mg/kg and 500mg/kg for a period of 4 weeks, while another group of rats without this treatment served as control. The diabetic control group showed significant ($P < 0.05$) increase in fasting blood glucose, but a decrease in serum C – Peptide and brain tryptophan levels compared with the control. Treatments with 375mg/kg and 500mg/kg nicotinamide showed a significant decrease in fasting blood glucose, but an increase in serum C-peptide and brain tryptophan levels compared with diabetic control. Data of the study indicate that nicotinamide may prevent diabetic complications by alleviating its metabolic symptoms of hyperglycemia and polyphagia, which may ameliorates pancreatic islet cell damage in diabetic rats.

Keywords*: Nicotinamide, serum C-Peptide, tryptophan, glucose, diabetes, rat.*

INTRODUCTION

Type 1 diabetes mellitus is an auto-immune disease characterized by destruction of the pancreatic islet cells, leading to a decrease in and eventually cessation of insulin secretion (Diabetes, 2014). Clinical overt Type 1 diabetes mellitus is thought to be a late stage of the process of gradual destruction of islet cells. It is possible to modify this process in order to slow down or prevent the development of clinical diabetes. Imperfection in insulin secretion results in increase production of glucose by the liver but decrease removal from the blood, leading to hyperglycemia, a clinical hallmark of diabetes mellitus (Banerjee *et al.*, 2005, Kentaro *et al.*, 1982). Hyperglycemia results in classic symptoms of polyuria, polydipsia and polyphagia. Hyperglycemia seems to initial a hunger signal that tiggers excess release of neuropeptide Y, resulting in polyphagia. Hyperglycemia exaggerates polyphagia (Diabetes, 2014). It is estimated that more than 300million people suffer from diabetes mellitus which has huge economic impact. A person with diabetes mellitus can face implication cost ranging from 1,000-15,000 dollars a year, such a serious drain on health resources in developing countries (Diabetes, 2014, Wahieb and Godin, 1987).. Hyperglycemia leads to acute and long-term metabolic complication of retinopathy, nephropathy, neuropathy and artherosclerosis which are significant source of morbidity and mortality. Insulin administration hardly prevent long-term complications of diabetes mellitus because optimal insulin dosage, which reduces complications, increases in risk of episodes of hypoglycemia and is difficult to adjust. In addition, insulin is also lipogenic (Steiner *et al.*, 1967). A clinical call to design a cost-effective, natural agent with hypoglycemia and ameliorating effects on diabetic complications has become medical priority (Welborn *et al.*,1981). Nicotinamide has been shown to prevent pancreatic islet from. Inflammation and to

be a component of glucose tolerance factor which enhances insulin sensitivity (Shibata, 1978, Ziegler, 2000). Large-doses of nicotinamide treatment reverse beta cell damage at the "honeymoon period" in diabetes (Trapp and Jung, 2006). The effect of nicotinamide on brain tryptophan in animal models has not been fully investigated (Freeman *et al.,* 2006). The objective of this study was to investigate the effects of nicotinamide on fasting blood glucose, serum C-peptide and brain tryptophan levels in STZ-induced diabetic rats and to suggest possible mechanism of action.

METHODS

Animals

Twenty male Sprague- Dawley rats (116.8±4.25g) were used for the study. The rats were acclimatized for a period of two weeks to the laboratory conditions in conformity with the international guidelines on ethics of animal experimentation. Rats were housed in well ventilated cages with 5 per cage at room temperature with 12h of light and dark cycle and access to drinking water and rat chow. The rats were fed with commercial rat chow and water *ad libitum.*

Induction of Diabetes

The rats were fasted for 24h before injection of freshly prepared solution of STZ was administered intra-peritoneally at a dosage of 55mg/kg body weight. STZ was freshly prepared in 0.1M sodium citrate buffer, pH 4.5. This dose produced type 1 diabetes having average fasting blood sugar level of 389.2±2.02mg/dl after 2days of injection.

Experimental Design

The rats were randomly divided into groups 1 and 2. Group 2 was later re-distributed into three groups with 5 animals per group in the whole.

Group 1 : Normal control rats were rat chow and water for 4 weeks
Group 2 : Diabetic rats were fed with rat chow for 4 weeks.
Group 3 : Diabetic rats received oral administration of 375mg/kg nicotinamide for 4 weeks.
Group 4 : Diabetic rats received oral administration of 500mg/kg nicotinamide for 4 weeks.

Four weeks after diabetic injection, the rats from each group were sacrificed after an overnight fast and blood samples collected from the eyes using capillary tubes. The blood samples were centrifuged at 2000rpm for 10min and the sera obtained were used for biochemical assay.

Biochemical Assays

Determination of serum C-peptide

The method is an immuno-enzymometric assay. The principle is based on antibody-antigen reaction. The test involves the linking of a labeled enzyme called conjugate (HRP-conjugate) to the test antigen (C-peptide) which is bound to a monoclonal biotylated antibody immobilized in a streptavidin coated microtiter well. The enzyme develops a yellow colored reaction which is measured spectrophotometrically at 450nm using ELISA reader (Irving *et al.,* 2005).

Determination of brain tryptophan

The method of Nkonge and Balance (1982), modified by Ebuehi and Akinwande (1992) was used to determine brain tryptophan concentration. This method is based on reaction between free tryptophan and ferric chloride solution under acidic conditions to produce a reddish yellow solution that is measured spectrophotometrically at 545nm.

RESULTS

The nicotinamide orally administered at 375mg/kg and 500mg/kg respectively decreased glucose levels in STZ-induced diabetic rats (Fig. 1). The free tryptophan concentration in the brain decreased in diabetic control compared with the normal control, but increased at 375mg/kg and 500mg/kg dosage respectively compared with the diabetic control (Fig. 2).
The bound tryptophan level in the brain decreased in the diabetic control but increased at doses of 375mg/kg and 500mg/kg compared with the diabetic control (Fig. 3). The effect of 55mg/kg body weight streptozotocin and treatments with 375mg/kg and 500mg/kg body weight on total tryptophan is presented in Fig. 4. The results show that 55mg/kg body weight significantly ($p< 0.05$) decreased brain tryptophan compared with normal control but treatments with 375mg/kg and 500mg/kg body weight significantly increased brain tryptophan compared with diabetic control.
The effect of 55mg/kg body weight streptozotocin and treatments with 375mg/kg and 500mg/kg body weight on fasting serum C-peptide is reported in Figure 5. The results show that 55mg/kg body weight significantly ($p< 0.05$) decreased fasting serum C-peptide compared with normal control, but treatments with 375mg/kg and 500mg/kg body weight nicotinamide significantly increased fasting serum C-peptide compared with diabetic control (Fig. 5).

DISCUSSION

The present study shows that nicotinamide possesses diabetic ameliorating potentials as evidenced by increased serum C-peptide and brain tryptophan with decreased fasting blood glucose levels in the treated diabetic rats compared with diabetic control. These parameters are indicative of increased insulin secretion and improved pancreatic islet function. Serum C-peptide has been shown to be bio-maker of insulin secretion. Dosing with C-peptide has been shown to improve diabetic complications. In this study, nicotinamide treatments resulted in increased serum C-peptide which may account for decreased fasting blood glucose and brain tryptophan levels in treated diabetic rats. The mechanism of action may be to repair the damaged pancreatic islet by enhanced poly-ADP- ribose polymerase activity. It has been shown that repair of beta cells by nicotinamide at the "honeymoon stage" restores pancreatic islet function. Studies reveal that nicotinamide increases the activity of poly-ADP- ribose polymerase activity. It is also suggested that nicotinamide upregulates poly-ADP- ribose polymerase level via chromatin remodeling. Studies have shown that nicotinamide activates NAD-dependent histone deacetylase which effect chromatin remodelin. Further studies to elucidate mechanism are required.

The significant increase in brain tryptophan implies "tryptophan sparing" in diabetic rats treated with nicotinamide. It has been shown that improved insulin secretion indication by increased C-peptide level facilitates brain tryptophan uptake by increasing skeletal muscle tissue uptake of competing aromatic amino acids. It also reduces brain tryptophan catabolism for energy production via increased glucose utilization. This "spares" tryptophan and increases its bioavailability for serotonin biosynthesis (Fernstrom and Wurtman, 1971, Ebuehi *et al.*, 2009). Studies have shown that serotonin is satiety-inducing neurotransmitter. In this study, nicotinamide treatment resulted in increase brain tryptophan level which may imply reduced polyphagia. The mechanism for increased brain tryptophan is via increased C-peptide secretion and improved pancreatic islet function (Welborn *et al.,* 1981). Further studies to elucidate mechanism are required. It is suggested that polyphagia exacerbates hyperglycemia which is the harbinger for diabetic complications such as retinopathy, nephropathy, neuropathy and atherosclerosis which are significant sources of morbidity and mortality. Nicotinamide treatment may prevent or delay the complications of diabetes by improving pancreatic islet function and increasing C-peptide secretion.

CONCLUSION

Data of the study indicate that nicotinamide treatment improved insulin secretion and pancreatic islet function in male rats after 28days. Furthermore, nicotinamide treatment improved brain tryptophan uptake and enhanced tryptophan sparing effect.

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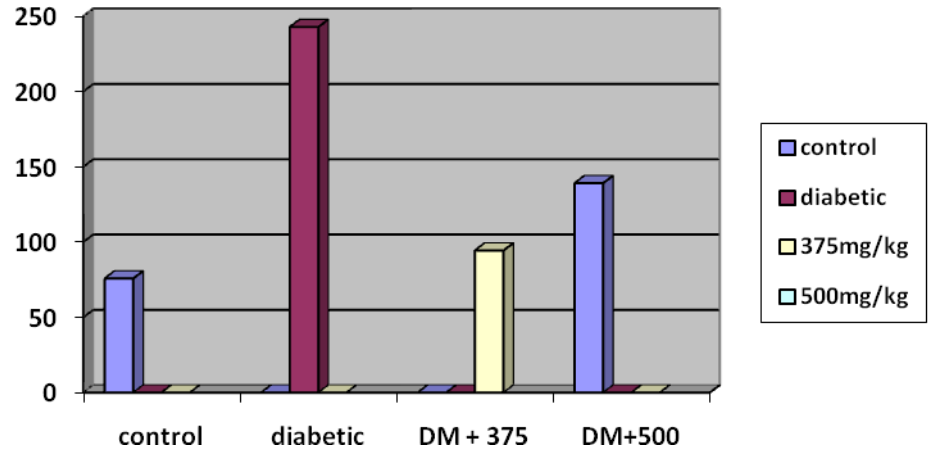

Figure 1: Effect of nicotinamide on fasting blood glucose in rat.

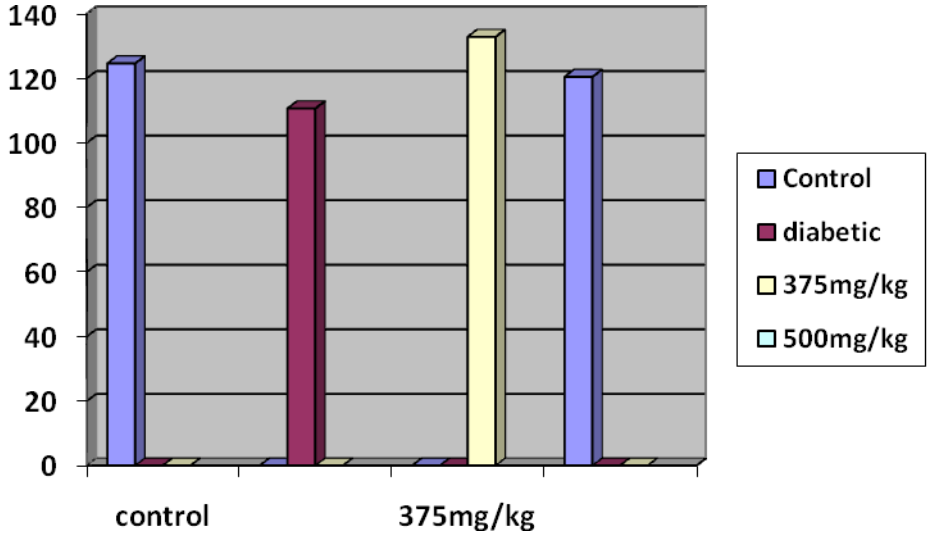

Figure 2: Effect of nicotinamide on free tryptophan in the brain.

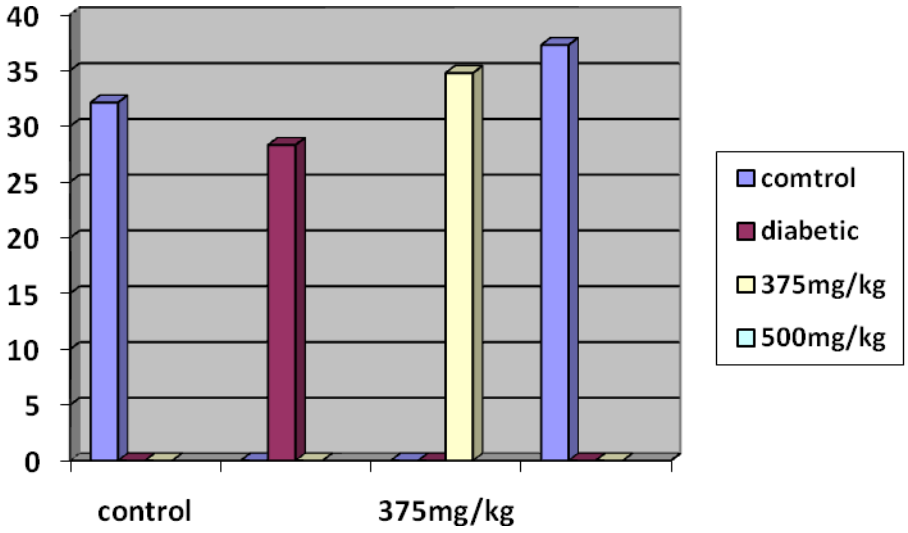

Figure 3: Effect of nicotinamide on bound tryptophan in the brain.

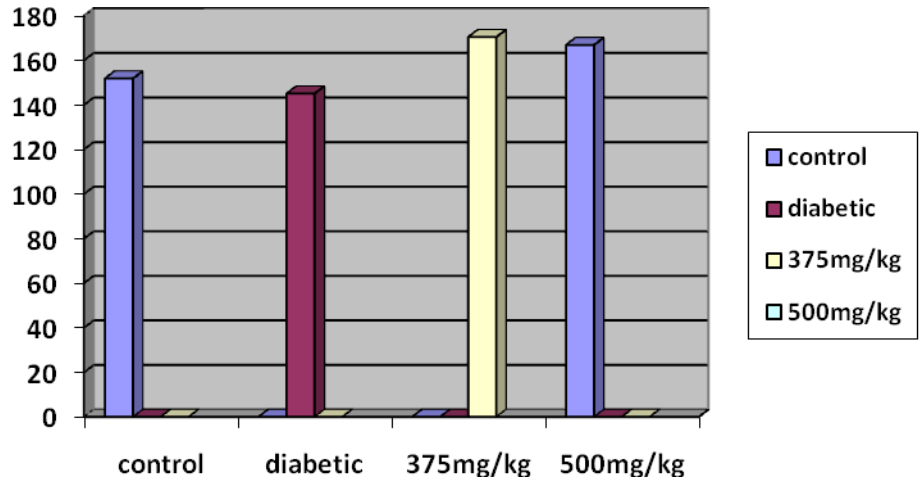

Figure 4: Effect of nicotinamide on total tryptophan in rat brain.

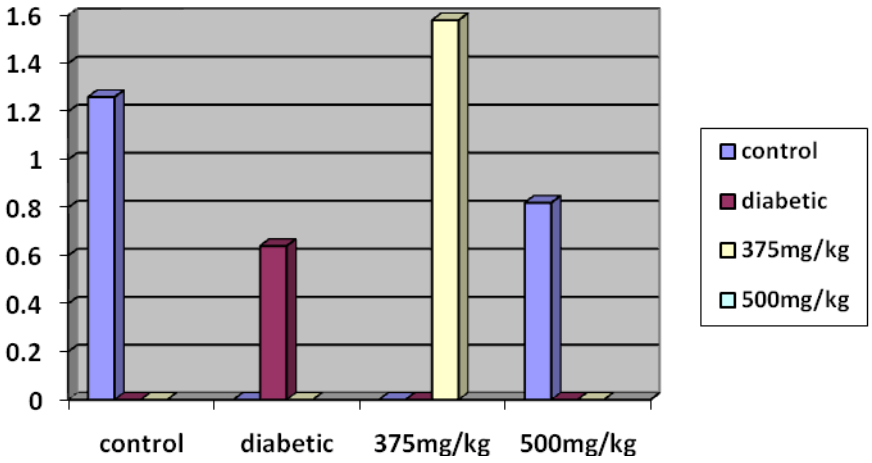

Figure 5: Effect of nicotinamide on serum C-peptide in rat.

PRELIMINARY REPORT ON ENVIRONMENTAL SOURCES *CLOSTRIDIUM DIFFICILE* IN LAGOS STATE, NIGERIA

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ABSTRACT
Clostridium difficile is the pathogen responsible for antibiotic-associated pseudo-membraneous colitis (Diarrhoea), which can be as a result of the use of broad spectrum antibiotics (such as ampicillin, clindamycin and the cephalosporins). These antibiotics can wipe away part of the normal intestinal flora allowing the pathogenic *Clostridium difficile* that is sometimes present to super infect the colon. When it grows in abundance, it releases its exotoxins. Toxin A causes diarrhea, and Toxin B is cytotoxic to the colonic cells. *Clostridium difficile* is known to cause severe diarrhoea, abdominal cramping and fever. In this study, a total of 480 environmental samples were collected from soil, water, vegetables, animal faeces and the hospital environments in five local government areas of Lagos State, Nigeria. With the Brazier CCEY Agar, *Clostridium difficile* spores were only found in the hospital setting examined. Seven (7) out of the Sixty (60) samples examined from the Medical Wards as well as three (3) out of the twenty (20) Intensive Care Unit (ICU) samples were *C. difficile* positive. In the medical wards, the areas examined were the patient bedsheets, bed railings, table top, toilets and windows. From the ICU, the spores were found on the drip stand, table tops and patient beds. However, *Clostridium difficile spores* were not isolated from the environmental samples examined. This finding suggests that *C. difficile* is more easily isolated in the hospital environment than the community. Therefore, more samples are still needed to be examined in more locations and other sources within the hospital as well as the community so as to improve our understanding about the ecology of *C. difficile* transmission.

Keywords: *Environmental, Clostridium difficile, Lagos*

INTRODUCTION
Clostridium difficile was once a relatively obscure organism to the general public but has recently gained prominence as a highly virulent intestinal microorganism capable of causing protracted hospital-associated diarrheal illnesses, especially in situations when antibiotics are administered (Taubes, 2008). Contributing factors to this greater awareness are a corresponding increase in the incidence of *C. difficile* infections (CDI), heightened severity of disease symptoms with higher reoccurrence rates, and an increase in antimicrobial resistance particularly in the United States (Wiegand *et al*., 2012).

The increase in CDI cases has spurred increased concern regarding the growing segment of community-associated infections distinct from infections acquired in healthcare settings (Limbago *et al*., 2009).

Importantly, some evidence has shown that *C. difficile* may be brought into the healthcare environment by asymptomatic carriers (Clabots *et al*., 1992). The reported carriage rates of *C. difficile* in healthy adults have varied from 0% to 3% in Europe to up to 15% in Japan (Mulligan, 2008).

However, little is known about the prevalence of *C. difficile* in the environment and how it may be transmitted to humans. In some environments, *C. difficile* has been found in a variety of environments, including water, soil, animal faeces, and foods (Al Saif and Brazier, 1996; Rodriguez-Palacios *et al*., 2007). These findings suggest that *C. difficile* may be transmitted to humans through food, although no foodborne cases have been reported. In previous studies, ready-to-eat foods have been implicated in foodborne disease outbreaks associated with *Salmonella* species and *Escherichia coli* O157 (Sagoo, *et al*., 2003 and Delaquis, *et al*., 2007)

In Nigeria, the distribution of *C. difficile* in the general environment is largely unknown. This study is aimed at investigating how healthy individuals in the community may be exposed to *C. difficile* spores and also to promote an understanding of the ecology of the organism in Lagos metropolis.

Several studies have reported the widespread presence of the organism in hospital wards and on the hands of nursing personnel (Macfarland *et al*.,1989). However, fewer studies have been directed at the environment outside the hospitals. Reports of *C. difficile* in the environment suggest either that its geographical distribution is not known or that different methodologies used were responsible for different isolation rates. (Al-Saif and Brazier, 1996)

For example, Hafiz from Sheffield, England in 1976 reported the organism in soil, sand and mud. Other workers in Korea and Poland have also found it in the soil. However, a study in Perth, Western Australia and a study of 20 random soil samples from Michigan, USA both gave negative results. (Kim, *et al*., 1981)

Furthermore, in a broad study carried out by Al-Saif and Brazier in 1996, only 184 out of 2580 samples representing about 7.1% yielded positive isolates from the domestic environment in Cardiff area of South Wales.

METHODS

Study Area

Water, soil, farm animal faeces and raw vegetables were collected from five (5) different Local government areas of Lagos State, Nigeria from the 27th-31st of August, 2012 using a simple stratified random sampling method.

CULTURE AND IDENTIFICATION

Processing of Water Samples

One Hundred (100) µL each of the water was filtered through 0.45µm filter membrane. The membrane filter was aseptically placed on the prepared Brazier's selective medium and incubated anaerobically at $37^{O}C$ for 48hrs in Anaerobic Jar (Biomerieux, France) containing N_2(80%), H_2(10) and CO_2 (10%). Anaerobiosis was achieved using anaerobic paper indicator which changes from blue to white in the presence of *Pseudomonas aeruginosa* ATCC 27853 obtained from the department of Medical Microbiology, College of Medicine of the University of Lagos. All culture plates were cultured for 48hrs and plates showing no growth were re-incubated for another 24hrs before being discarded. All isolates grown were tested with metronidazole antibiotic disc. The isolates that show Gram positive rods with spores were

presumptively identified as *Clostridia* and stored in 15% anaerobic glycerol broth in -80^{o}C for further identification. **(**Al Saif and Brazier, 1996)

Processing of Soil/Animal Faeces

All soil and animal faecal samples were mixed vigorously in the same ratio with 15% methyl alcohol and left to stand for 30 minutes to knock-out all vegetative cells leaving only the spores. The mixtures was then allowed to settle and a loopful of the deposit was aseptically streaked on already prepared Brazier CCEY medium and incubated anaerobically at 37^{O}C for 48hrs in Anaerobic Jar (Biomerieux, France) containing N_2(80%), H_2(10) and CO_2 (10%). All plates showing no growth were further re-incubated for another 24 hrs before being discarded.

Processing of Vegetables

The unwashed surfaces of the samples was placed directly on the medium and then discarded. Common local vegetables samples such as ugwu leaves, ewedu, efo tete, onions and tomato were used. Anaerobic incubation was carried out as described for the above samples**.(**Al Saif and Brazier, 1996)

Biochemical Identification

Biochemical identification of *C. difficile* was carried out using API 20A (BioMerieux) Identification System. The system enables 21 tests to be carried out quickly and easily for the biochemical identification of anaerobes. Other test such as colonial and microscopic morphology, Gram stain was performed and the results used to complete the identification.

RESULTS

A total of 480 samples were collected for this study from the environment. These include soil, water, vegetables, animal faeces and the hospital environment. *Clostridium difficile* spores were only found in the hospital setting examined. Seven (7) out of the Sixty (60) samples examined from the Medical Wards as well as three (3) out of the twenty (20) Intensive Care Unit (ICU) samples were *C. difficile* positive. In the medical wards, the areas examined were the patient bed sheet, bed railings, table top, toilets and windows. From the intensive care unit (ICU), the spores were found on the drip stand, hospital floors, table tops and patient beds. It was observed from the medical records that patients in both sections of the hospital (medical wards and ICU) are on heavy antibiotic use. Four (4) out of the Five (5) patients observed in the ICU were on post-surgery treatment while one (1) was reported to be recovering from complications arising from a caesarian operation as a result of prolonged labour. The patient was also observed to be passing loose stool; an indication of a possible diarrheal infection.

In the medical ward (A3), a patient was seen passing stool while some others were seen passing urine in plastic containers brought by the health attendant who later disposed it in the toilet. This can be a potential way in which *Clostridium difficile* spores may escape into the surrounding environment if it had already colonized the gut of the patients.

The result from the hospital setting was not unexpected as *C. difficile* has been demonstrated to cause a health-care associated infection. There were no *C. difficile* spores in the soil, water, vegetables and animal faeces but other *Clostridia* species were found in them.

Table 1 shows the summary of results obtained for the Identification of *Clostridia* species from the samples analysed. In the soil, water, animal faeces and vegetables samples examined, no *C. difficile* was identified but other Clostridia species were found in some of the samples.

DISCUSSION

This study investigated the presence of *C. difficile* spores in the environment. It was observed that *C. difficile* spores were only found in the hospital environments (the medical wards and intensive care unit) but were not found in the environmental samples examined (water, soil, animal faeces, and vegetables).

Clostridium difficile was isolated from seven (7) out of the sixty (60) samples examined from the medical wards from the patient bed, floors, toilet and windows. Patients' medical record obtained from the medical registry showed that the patients were all above 50 years of age, on heavy antibiotic use while others have stayed in the hospital for close to two (2) months. Some of the patients were seen passing stool and urine in a plastic container provided by the hospital health attendant before being disposed in the toilet. This can be a means of *C. difficile* spore transmission to the surrounding environment if the patients' guts have been colonized with *C. difficile* spores.

In the Lagos University Teaching Hospital-Intensive Care Unit (LUTH-ICU), three (3) out of the twenty (20) samples examined from the drip stand, bed rails and table top was identified as *C. difficile* positive. At the time of examination, record shows that four (4) out of the five (5) patients have been admitted for about seven (7) weeks, on heavy drug administration and above 55 years of age. These are some of the identified risk factors for *C. difficile* colonization in the hospital. In addition, one of the patients had medical complications arising from caesarian operation after prolonged labour. The patient was reported to be passing loose stool. This may be an indication that she had diarrhea.

Furthermore, the result obtained in this research from the hospital setting was not unexpected as *C. difficile* has been demonstrated to cause a health-care associated infection.
Several studies have reported the widespread presence of *C. difficile* in hospital wards and on the hands of nursing personnel (Kim *et al*., 1981; Macfarland *et al*., 1989; Egwuatu, 2011, unpubl).
Macfarland *et al.,* (1989) reported that the study of the epidemiology of *C. difficile* has identified both asymptomatic *C. difficile* culture-positive patients and contaminated environments as potential sources of diarrheal outbreaks. Some other studies have reported the environment surrounding both symptomatic and asymptomatic patients as reservoir for *C. difficile* which is potentially transmitted by contact with fomites, staff and other patients.

Best *et al*., (2010) informs that that though the spores of *C. difficile* can occasionally spread through the air but can be easily transmitted through the hands of hospital staff.
Studies conducted by Titov, *et al* (2000); Fawley and Wilcox, (2001) and Egwuatu, (2011) all confirmed and supported that in the hospital particularly the ICU, strains of *C. difficile* may be introduced by hospitalized patients or staff and transmitted to other patients and their environment.

In another study carried out in Kuwait, Rotimi and other researchers (2002) reported that the acquisition rate of *C. difficile* increased from 5.9 to 36% during 4 to 53 days of hospitalization in various wards.

From the results obtained in this study, 7 out of the 60 samples examined in the medical wards were *C. difficile* positive. A recovery rate of 12.5% from the LUTH hospital environments suggest that the main route of transmission may be through hospitalized patients. In addition, the low rate of recovery (12.5%) may be as a result of the wards sampled, the underlying medical

conditions of the in-patients, the environment, the staff as well as the technique of sampling employed.

In another study by Conly (2000), the risk of colonization by *C. difficile* was found to increase in a direct proportion to the length of hospital stay ranging from 13% among patients admitted for less than 1 week to as high as 50% among patients admitted for more than 4 weeks; this suggests that exposure to *Clostridium difficile* occur throughout the hospital stay.

In another research conducted by Akhi *et al*., (2011), out of 70 *C. difficile* isolates which were cultured as a first time in North West Iran, 18% (18/100), 10.37% (14/135), 32% (16/50) and 44% (22/50) were isolated from staff, hospital environment, patients at first day of admission and the same patients after seven days of hospitalization respectively. Six patients (12%) were reported to be colonized by *Clostridium difficile* during days of hospitalizations.
Fourteen isolates (10.37%) of *Clostridium difficile* was obtained from various region of pulmonary (n=5), infectious disease (n=3), ICU of neurology (n=3), ICU of pulmonary (n=2) and endocrine and rheumatology (n=1) wards.

In this study, no *C. difficile* was identified from the environmental samples but were found in the hospital environment. For instance in a broad study carried out by Al-Saif and Brazier (1996) using a total of 2,580 samples, only 184(7.1%) yielded *C. difficile* from samples obtained from the hospital environment as well as some environmental samples. From their study, it was suggested that the non-isolation of *C. difficile* from the environment may be due to their geographical distribution or by the cultural methodology applied which may cause different isolation rates. This shows that the isolation of *C. difficile* in the environment may be very difficult to establish.

Furthermore, a study in Perth, Western Australia and a study of 20 random samples from Michigan, USA (Kim *et al.,* 1981; Riley 1994) both gave negative results for the isolation of *C. difficile*. All these further confirm the result obtained in this present study.
The results of this study also suggest that the consumption of Vegetables is not likely to be an important source of exposure to the bacterium. In addition, other samples such as water, animal faeces and soil appear seem to have no or low risk potential as no *C. difficile* was identified from them.
The presence of other *Clostridia* species in water samples has been demonstrated in several studies (Davies 1969). If *C. difficile* were to be present, it shows the potential of the water supplies to be a source of infection in case of treatment failure.

Clostridium perfringes was isolated from the water samples examined. This finding is in agreement with previous studies which show that their presence is thought to indicate a failure in the water filtration process. The study showed that fifty (50) out of fifty-five(55) anaerobic bacilli was isolated from sand filters in sand beds to show that they could be reservoir of *Clostridia* species (Nankiveli, 1911). In Addition, it could be as a result of faecal contamination of the water source and may be a potential source of *C. difficile* in the near future.

In Nigeria, there is minimal literature and research on the transmission of *Clostridium difficile* from hospital staff to patients and none looking solely at doctors but the hands of hospital personnel caring for patients with *C. difficle* often become colonized with the bacterium thereby facilitating transmission among hospital in-patients. Therefore, more studies are still needed to be carried out in this regard to ascertain their level of transmission.

CONCLUSION

In the Lagos population examined, *C. difficile* was only found within the hospital setting but was not identified from the environmental samples. This suggests that *C. difficile* is more easily isolated in the hospital environments than the general environment. More samples are still needed to be taken in more locations and other sources within the hospital as well as the larger community to improve our understanding of the ecology of *C. difficile* transmission.

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Table 1: Summary results for the Identification of Clostridia species from Environmental Samples

Source	n	Local Government Areas	Number(%) samples positive
Soil	100	Agege (10), Ojo (9), Epe (10)	29(29)
Animal Faeces	100	Epe(20), Agege (10), Epe (10) Lagos Mainland (20)	60 (60)
Water Samples	100	Epe(11) Oshodi (10) Agege (10)	31(31)
Vegetables	100	Epe (20),Agege (20), Ojo (20), Oshodi (20) &Lagos Mainland (20)	0(0)
Hospital Environment		Medical Wards & ICU	10 (12.5)
Total		**480**	**130(27.1)**

N: number of samples

EFFECTS OF EXERCISE TRAINING ON SELECTED CARDIO-PULMONARY PARAMETERS AND BODY COMPOSITION OF NIGERIANS WITH BI-VENTRICULAR HEART FAILURE
(A PRELIMINARY STUDY)

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ABSTRACT
Background: The use of exercise training in the management of individuals with chronic heart failure has not been widely accepted by health care providers especially in Sub-Saharan Africa because of the possibility that the failing hearts may have a negative response to the increased workload and stress of exercise. **Objective:** The study aimed to evaluate the effects of exercise training (ET) on selected cardio-respiratory and body composition variables of Nigerians with CHF. **Methods:** Thirty two Nigerians with chronic heart failure (53% male), aged 30 to 71 years, mean age 54.2±1.9years and New York Heart Association Functional Class (NYHA) 11-III (a classification of patients with cardiac disease based on clinical severity and prognosis) recruited from Cardiology Unit of Lagos University Teaching Hospital Nigeria participated in the study. They were randomized into exercise and control groups. Exercise group performed 12- week ET for 60 minutes three sessions per week. Selected cardio-respiratory and body composition variables were measured pre and post intervention in both groups. Data was analyzed using SPSS-17 package. **Results:** There was no significant difference in the measured variables between the groups at baseline. Significant improvement was seen in EG in all the measured variables except the systolic and diastolic blood pressure. No significant improvement was observed in the control group. **Conclusions: E**xercise training may improve cardio-respiratory and body composition variables in CHF.

Keywords: *Exercise training, Chronic heart failure, Blood pressure, Body composition.*

INTRODUCTION
Heart failure (HF) is an endemic syndrome and constitutes an important public health problem around the world[1]. In the United States, HF affects about 5 million people, and 550,000 new cases are diagnosed every year[1]. In developing countries, it accounts for about 4% of hospitalizations and 31% of those are due to heart and circulatory illnesses. Furthermore, it has a high mortality rate (20%) and it is estimated that, after diagnosis, only 15% of patients are still alive after 12 years (Hunt *et al*., 2009).

The inability to perform exercise without discomfort is one of the first symptoms experienced by patients with HF (Benno *et al*., 2003). The number of people with HF is rising yearly and many patients suffer from dyspnoea, fatigue, diminished exercise capacity and poor quality of life (Benno *et al*., 2003). In the past, individuals with HF were advised to avoid physical exertion in the hope that bed rest might minimize symptoms (McDonald *et al*., 1972) and in the belief that physical activity might accelerate the progression of left ventricular dysfunction (Jugdutt *et al*., 1988). Various drugs have been developed in order to control hemodynamic disorders and symptoms, as well as to reduce mortality and improve the quality of life of heart failure patients. In spite of a large number of pharmacological agents that successfully decrease mortality, the

effects on exercise tolerance are modest, even patients with guideline-based optimized therapy often remain limited by their symptoms and, depending on their conditions, their functional capacity and quality of life may be substantially reduced (Dubach *et al*., 2001,Cowie & Zaphirou, 2002) .

However, in recent years it has been suggested that exercise therapy is crucial and effective in the management of cardiovascular disease (Lamina & Okoye, 2010). Though many studies have demonstrated improved systolic and diastolic function in heart failure patients in response to exercise training (Belardinelli *et al*., 1995, Gianuzzi *et al*., 2003, Stolen *et a*l., 2003), Blacks and other racial minorities with HF are under-represented in most of these clinical trials of HF, which compromises the extrapolation of results from major clinical trials to ethnic subgroup populations. . .It is also believed that exercise restriction may lead to de-conditioning and increased morbidity (Sullivan et al., 1989, Coats et al., 1992, Belardinelli *et al*., 1995, Meyer 2001, Cowie & Zaphirou, 2002, Anigbogu & Ajiboye 2010). The majority of studies in this area were done on Caucasians whose aetiology are mostly ischaemic, there is paucity of evidence based studies in Sub-Sahara Africa especially in Nigeria among HF population whose aetiology is mostly hypertensive. The aims of this study were to evaluate effects of exercise training on selected cardio-respiratory functions (systolic and diastolic blood pressure, heart rate, oxygen saturation (SpO_2), resting respiratory rate, resting metabolism and Body composition (% body fat, % body muscle) of individuals with CHF.

METHODS

Subjects' selection

Thirty two (32) CHF patients (17 male (53%)) who were receiving maximal pharmacological treatment in Cardiology Unit of Lagos University Teaching Hospital, Nigeria participated in the study. They were in Class II and III of New York Heart Association (NYHA) classification. The study was carried out in the medical gymnasium of Physiotherapy Department, Lagos University Teaching Hospital, Lagos. Nigeria.

New York Heart Association Classification:

1. Class I: no limitation is experienced in any activities; there are no symptoms from ordinary activities.
2. Class II: slight, mild limitation of activity; the patient is comfortable at rest or with mild exertion.
3. Class III: marked limitation of any activity; the patient is comfortable only at rest.
4. Class IV: any physical activity brings on discomfort and symptoms occur at rest.

(AHA, 2002)

Ethical Consideration

The protocol for this study was approved by the Health Research and Ethics Committee of the Lagos University Teaching Hospital (LUTH), Idi-Araba, Lagos (Appendix 1) before the commencement of the study. Written consent was also obtained from subjects before enrolment into the study.

Inclusion Criteria

They were oriented to person, place, and time. stable CHF in class II and III of NYHA classification, no change in drug therapy for 30 days; resting ejection fraction ≤40% as measured by echocardiography, medical diagnosis of heart failure either hypertensive or idiopathic in origin. They were receiving standard pharmacologic therapy for heart failure (diuretics, ACE inhibitors, β blockers and digoxin) and not previously engaged in structured exercises in the past six months. Individuals with no musculoskeletal limitations such as rheumatoid arthritis, severe

osteoarthritis, and other joint problems that could limit their exercise performance were included in this study.

Exclusion Criteria

Patients with any of the following medical conditions were excluded from the study: atrial fibrillation,acute heart failure within the previous 3 months, unstable angina pectoris,ischaemic HF, end-stage renal disease, orthopaedic impediments to exercise, subjects participating in a formal exercise program within 6 months before this study.

Measurements

Subjects' height and weight were measured usingstandiometer (Seradon, England). Age was recorded in years. Body weight, BMI, % body fat, % body muscle and resting metabolism of subjects were measured by Karada Scan Body composition monitor BF 511 (Omron Netherland).Blood pressure was measured with Omron automatic blood pressure monitor (Bannockburn, Illinois). Polar heart rate monitor was used to monitor the heart rate during the exercise. The BF511 was used to measure body fat percentage by the bioelectric impedance (BI) method. The modified Borg's rate of perceived exertion was used as a check tool; subjects were instructed to indicate how they feel based on the scale. They were encouraged to exercise at moderate intensity range at point 3 – 4 of the scale which denoted "moderate to somewhat hard". The control group participated in measurements only and they were told not to initiate or participate in any structured exercise training.

Exercise Training Protocol

Exercise training was performed 3 times weekly for 3 months. Each session included 10 minutes of warm-up, 20 minutes of cycling (20Watts) and20 minutes of resistance training at 50% of 1RM for three sets of 10 repetitions of four major muscle groups in both upper and lower limbs (elbow flexion, knee flexion, knee extension and hand grip exercises).Each exercise session was concluded with 10 minutes of cool- down exercises. The warm-up and cool-down exercise comprised of mild aerobic exercises, stretching and flexibility exercises. They performed three sets each of four common resistance training exercises; 50% of 1RM for 10 repetitions per set from 1^{st} to 6^{th} week, 60% of 1RM for 10 repetitions per set from 7^{th} to 12^{th} week. The loading on the ergometer was also progressed by 5Watts individually depending on their responses to treatment at the end of each month.

Data Analysis

Analysis of the socio-demographic data was done using descriptive statistics of mean and standard error of mean. Paired t test was used to compare pre- and post-tests of the subjects in EG and CG at baseline and the end of 12 weeks. Independent t test was used to compare variables between EG and CG. Level of significance was $p < 0.05$.

RESULTS

All the patients that met inclusion criteria were 132 (male- 69, female - 63) out of which only 48 gave their consents, 38 reported for the study but only 32 completed the study with attrition rate of 15.8%. Seventeen (17), 54.5% had biventricular heart failure secondary to hypertension while fifteen (15), 45.5% had biventricular heart failure secondary to dilated cardiomyopathy (DCM).

The mean age, height , weight and body mass index of subjects in control group was(53.7 ± 3years, 1.65 ± 0.02 m, 74.1 ± 3.6kg, 27 ± 1.9 kg/m^2) respectively while that of exercise group was (54.1 ±2.2yrs,1.66 ± 0.02 m,79.2 ± 3.1kg,30.4 ± 1.3kg/m^2) respectively as shown in Table 1. There was no significant difference in their age, height, weight and BMI ($p = 0.901$, 0.886, 0.288 and 0.389) respectively.

Table 2 shows RHR, SBP, DBP, RRR and SPO_2 of the two groups in pre- and post tests.
In the exercise group, there were significant reduction in the RHR (p = 0.000) and RRR (p = 0.000) respectively and significant increase in % SPO_2 (p = 0.005). There were reduction in the SBP and DBP but the reduction was not significant after the 12weeks of aerobic and resistance training.
In the control group, there was a slight increase in RHR and SBP though the increase was not significant while significant increase was observed in RRR (p = 0.133).Slight reduction was also seen in SPO_2 within the group (p = 0.504).

Table 3 shows % body fat, % body muscle, resting metabolism and body mass index of subjects in exercise and control groups pre and post tests. In the exercise group, significant increase was observed between the pre and post test in the % body muscle (p = 0.001) while significant reduction were observed in % body fat (p = 0.001) and resting metabolism (0.018). In the control group no significant difference was observed between the pre and post test in all the measured variables.

Figure (A) shows the comparison between change in resting metabolism, percent body fat and percent body muscle in the control and exercise groups after the study. Significant reduction was observed in the change in resting metabolism and percent body fat of the subjects in exercise group as compared with the control (p < 0.001). Significant improvement in the change in percent body muscle was also observed in exercise group compared with control group (p < 0.001).

DISCUSSION

The aim of this study was to evaluate the effects of exercise training on the cardio-respiratory and body composition variables of Nigerians with chronic heart failure.

Effects of Exercise Training on Resting Heart Rate (RHR)

In this study, mean RHR of the exercise group was reduced by 11.3beats/min while that of the control group was increased by 0.9beat/min. There was a significant difference between RHR of two groups, after 12 weeks. Also, this finding shows that the resistance and aerobic exercise training may have a significant effect in reducing resting heart rate in this population. This finding is in agreement with others (Roveda *et al*., 2003, Flynn *et al*., 2009) which demonstrated the beneficial effects of enhanced vagal tone and decreased sympathetic tone with exercise training. The mechanism for reduction of RHR can be due to improvement in the vagal tone and reduction in sympathetic tone. The decrease in sympathetic tone will not only decrease the occurrence of arrhythmias, but will lower heart rate both at rest and during exercise. Therefore, an endurance-trained heart operates at a lower heart rate, demanding less oxygen. The result is a heart muscle with a higher capacity that is better adapted to handle the rigors of life. The improvement observed may also be associated with peripheral changes such as an increase in systemic AV O_2 difference, with improved leg blood flow and a reduction in arterial and venous lactate levels, as reported by other investigators (Sullivan *et al.*, 1988, Coats *et al*., 1992, Shemesh *et al*., 1995). The result of this study further supports that exercise training may ameliorates neurohormonal abnormalities seen in heart failure patients with resultant reductions in heart rate, increased heart rate variability, and declines in sympathetic nervous activity.

Effects of Exercise Training on Blood Pressure

In this study, exercise training brought about reduction in both systolic and diastolic blood pressure though this reduction was not significant. After 12-week of training systolic and diastolic blood pressure had a 6.8 mmHg and 3.4mmHg decrease in the exercise group and

2.2mmHg increase in SBP and 0.5mmHg increase in DBP in the control group. This finding is not in agreement with the result of Whelton *et al*., (2002) who reported that among three ethnic groups, black participants had significantly greater reductions in SBP and Asian participants had significantly greater reductions in DBP compared with white participants. The contradiction of this result with the previous study may be related to the study population. They studied hypertensives while this present study was on heart failure. Kelley (1999) reported a small but significant reduction of blood pressure in studies that only involved women, all of whom were normotensive at baseline.

Effects of Exercise Training on Resting Respiratory Rate

In this study, combination of aerobic and resistance exercise had significant reduction in the resting respiratory rate in the exercise group. The shortness of breath that CHF causes is very debilitating; it deters exercise and makes it un-enjoyable. In a healthy individual, the lungs are well adapted and are never the limiting factor during maximal exercise. However, in a CHF patient, the fluid buildup in the lungs severely impairs breathing and limits the patient's exercise ability. Both biochemical and functional abnormalities in skeletal muscle are often present, limiting muscle metabolic capacity including reduced respiratory muscle endurance which may be present as part of the generalized skeletal myopathy in patients with CHF. This abnormality may contribute to the symptoms of fatigue and dyspnoea on exertion. The diaphragm shows a different adaptation from skeletal and respiratory muscle. There is a shift from fast to slow fibers with an increase in oxidative capacity and a decrease in glycolytic capacity (Tikunov *et al*., 1997). There are also changes in pulmonary function in CHF. Even in the absence of pulmonary congestion, CHF is associated with impaired pulmonary diffusion (Smith *et al*., 1999). and an exaggerated increase in minute ventilation in response to exercise, out of proportion to the increase in carbon dioxide production (Sullivan *et al*., 1988, Buller *et al.*, 1990). Hyperventilation is primarily due to ventilation/perfusion mismatching, the severity of which is related to the severity of the heart failure (Buller *et al*., 1990).

These changes are similar to those seen in the limb muscles that occur with endurance training, suggesting that they result from the increased work of breathing. Additionally, because the heart is so stretched out in CHF patients, it has an impaired cardiac output and struggles to pump adequate blood to the lungs for oxygenation. As a result, oxygen saturation of the blood drops. The finding is in agreement with the work of Mancini *et a*l 1995 who reported improvement in the strength and endurance of the muscles of respiration, relieving dyspnoea during exercise and even during rest which was associated with an increase in mitochondrial density, which reflects an improvement in oxidative capacity of trained skeletal muscles. The resulting effect is that the lungs are relieved of their burden of ventilation and the patient feels less of an urge to breathe at a given level of exertion, thus relieving dyspnea (shortness of breath).

Effects of Exercise Training on Body Composition

The result of this study shows significant increase between the pre and post test in the % body muscle ($p = 0.001$) and significant reduction in % body fat ($p = 0.001$) and resting metabolism (0.018) in the exercise group. In the control group no significant difference was observed between the pre and post test in all the measured variables. This result supported the findings of Benno et al (2003), they reported increased resting metabolic rate in heart failure and associated this with probably to what contributed to the cause of cardiac cachexia which is characterized by a negative energy balance and subsequent weight loss and systemic wasting that occurs frequently in patients with end-stage heart failure. The mechanism for the higher resting metabolic rate in patients with heart failure is unknown, but this may be attributed to an increased myocardial oxygen requirements and the increased metabolic cost of breathing. The

good news is that the result of this study was able to reduce the resting metabolism and reduce the resting respiratory rate of Nigerians with heart failure. Significant increase in % body muscle and reduction in % body fat may translate to increase strength and improved QoL. Exercise training was able to reduce the resting metabolism and the resting respiratory rate of Nigerians with heart failure. Significant increase in fat free mass and reduction in fat mass observed may translate to increase strength and improved QoL since body fat beyond normal reported range represents an excess burden on an already limited cardiopulmonary system and is associated with decreases in functional capacity.

CONCLUSION

It can be concluded from this study that exercise training reduces resting heart rate, resting respiratory rate, percentage body fat, improve the resting oxygen saturation and percentage body muscle in Nigerians with chronic heart failure.

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APPENDIX 1

Table 1: Physical Characteristics of subjects in Exercise and Control Groups

Physical Characteristics	Control Group Mean ± sem	Exercise Group Mean ± sem	P-Value
Age (years)	53.7 ± 3	54.1 ±2.2	0.901
Height (meters)	1.65 ± 0.02	1.66 ± 0.02	0.886
Weight (kilograms)	74.1 ± 3.6	79.2 ± 3.1	0.288
Body mass index (kg/m^2)	27 ± 1.9	30.4 ± 1.3	0.389

$P < 0.05$

Table 2: The mean values of RHR, SBP, DBP, RRR and SPO_2 variables in exercise and control groups in pre and post test.

Groups	Control group			Exercise Group		
Variables	Pre-test Mean± SEM	Post-test Mean± SEM	p-value	Pre-test mean± SEM	Post-test Mean ± SEM	p-value
RHR (bpm)	89.7± 3.5	90.6± 2.7	0.927	87.2± 2.0	75.9 ±2.5	<0.001**
SBP (mmHg)	120.5± 4.6	123.9± 3.6	0.186	126.9 ± 8.7	120.7 ± 4.7	0.296
DBP (mmHg)	78 ± 4.2	80.3± 3.8	0.128	81.9 ± 3.4	78.2 ±2.4	0.451
RRR (rpm)	22.7 ± 1.6	24.2± 1.5	0.133	26.4 ± 1.2	21.6 ± 1	<0.001**
SPO_2 (%)	97.3 ± 0.3	97.2± 0.3	0.504	96.4 ± 0.5	98.3 ± 0.2	0.005

RHR- resting heart rate; SBP – Systolic Blood Pressure; DBP- Diastolic Blood Pressure; RRR- resting respiratory rate; SPO_2 – Oxygen Saturation.
$P < 0.05$.
** = $p<0.001$

Table 3: Body Composition of Subjects in Exercise and Control Groups

Groups	Control	Group		Exercise	Group	
Variables	Pre-test Mean ± sem	Post-test Mean ± sem	P-Value	Pre-test Mean ± sem	Post-test Mean ± sem	P-Value
Body fat (%)	33.5 ± 3.8	33.9 ± 3.9	0.182	40.7 ± 3	38.5 ± 2.8	0.001

Fat mass ()	23.8 ± 3.7	24.1 ± 3.6	0.458	32.5 ± 2.6	30.6 ± 3.2	0.034
Body muscle (%)	27.8 ± 2.6	27.5 ± 2.6	0.375	24.5 ± 1.7	26 ± 1.5	0.001
Muscle mass ()	20 ± 1.8	19.7 ± 1.8	0.202	19.9 ± 1.7	20.9 ± 1.3	0.043
Resting Metabolism (kcal.)	1451 ±55.7	1448 ± 53	0.607	1589 ± 61	1533 ± 55	0.018
Body Mass Index (kg/m^2)	27 ± 1.9	26.9 ± 1.9	0.781	30.4 ± 1.3	29.8 ±1.1	0.118

P< 0.05

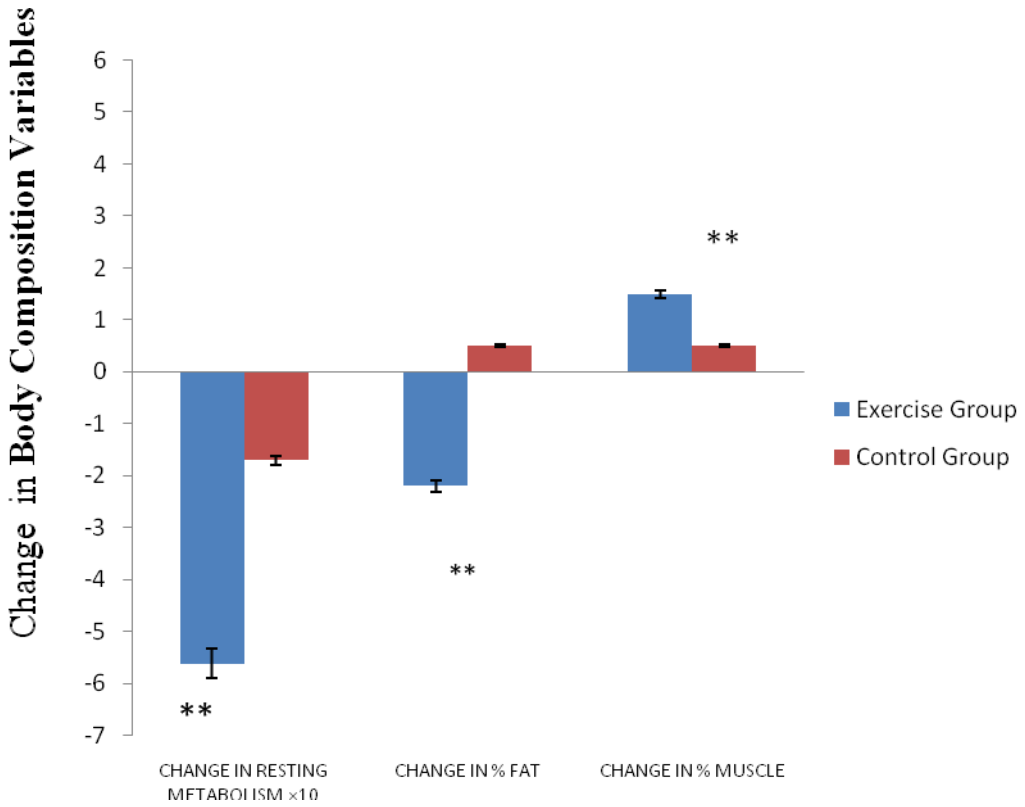

Figure A: Change in % body fat, % body muscle and resting metabolism in the EG and CG after 12-week exercise training

BURDEN OF PERIPHERAL ARTERIAL DISEASE (PAD) AMONGST PATIENTS WITH DIABETES MELLITUS FOOT SYNDROME (DMFS) IN LAGOS, NIGERIA

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ABSTRACT
Background: Worldwide, foot ulceration is a major cause of morbidity and mortality in patients with diabetes mellitus (DM). It occurs against a background infection, neuropathy and peripheral arterial disease (PAD). Inadequate evaluation of these factors can negatively impact on the treatment and outcome of diabetes mellitus foot syndrome (DMFS). Peripheral arterial disease is frequently detected solely by the palpation of the pedal pulses in resource poor settings. This method has however been found to underdiagnose PAD. On the other hand, the use of a hand held Doppler to calculate the ankle brachial index (ABI) is a simple, inexpensive, non-invasive and quantitative method of determining the presence of PAD. **Aim:** To determine the prevalence of peripheral arterial disease (PAD) in patients with DMFS in a tertiary health care centre using a hand held Doppler to determine the ABI. **Methods**: A retrospective study of patients with DMFS who had assessment for PAD using a hand held Doppler was done. The data retrieved from the doppler report included their age, gender, presence of hypertension, smoking status and ankle brachial index (ABI). Peripheral arterial disease was defined as the presence of ABI of < 0.9 or >1.3. Data was analyzed using Epi info version 3.4.3. Statistical significance was set at $p<0.05$. **Results**: There were 73 patients with DMFS, 28 (38.4%) females and 45 (61.6%) males. The mean (SD) age of the study participants was 62.4 (11.1) years. Hypertension was present in 30(41.1%) of the patients and 8(11.0%) were smokers. Peripheral arterial disease was present in 39(53.4%) patients, comprised of 17(43.6%) females and 22(56.4%) males. Of the 39 patients with PAD, 11 (28.2%) had ABI >1.3. Age ($p=0.143$), gender ($p=0.457$), smoking status ($p=0.607$) and history of hypertension ($p=0.898$) were not associated with the presence of PAD. **Conclusion**: The burden of PAD amongst these patients was relatively high (being 53.4%) and asignificant proportion(28.3%) had ABI>1.3. Hand held Doppler devices should be made readily available, at least in tertiary hospitals in resource poor settings for the better management of patients with DMFS.

Keywords: *peripheral arterial disease, ankle brachial index, diabetes, foot, ulcer, Lagos, Nigeria.*

INTRODUCTION

Worldwide, foot ulcers are a major cause of morbidity and mortality amongst people with diabetes.(Ekpebegh et al., 2009) Up to 25% of patients with diabetes will develop a foot ulcer in their lifetime.(Singh et al., 2005) Peripheral arterial disease (PAD) is one of the most important risk factors for developing diabetes mellitus foot syndrome (DMFS). The presence and severity of PAD impacts negatively on healing rates of DMFS, and is associated with major amputation and mortality amongst these patients. (Brechow et al., 2013) Peripheral arterial disease is also frequently accompanied by other life threatening complications of diabetes such as cerebrovascular disease and ischemic heart disease.(Alzamora et al., 2013; Allison et al., 2008) Thus, identification of and adequate assessment for PAD amongst patients with DMFS is of utmost importance.

Clinical assessment for PAD includes a history of intermittent claudication and reduced/absent

lower limb pulsation on palpation. Commonly used non-invasive methods for investigating PAD include magnetic resonance angiography, computer tomography angiography, colour duplex scanning and ankle brachial index (ABI) using a hand held doppler. The first four methods are expensive and not readily available in resource poor settings. Hence, PAD is often diagnosed solely by clinical evaluation in such situations.(Edo et al., 2013; Nyamu et al., 2003) Clinical assessment for PAD has its own limitations, being subjective and shown to underestimate PAD.(Ikem et al., 2010) The use of a hand held Doppler to derive the ABI is a simple, inexpensive, non-invasive and quantitative method of determining the presence of PAD.(Marso and Hiatt, 2006) The ankle brachial index is the ratio of the systolic blood pressure (SBP) at the ankles (posterior tibial and dorsalis pedis arteries) to the SBP at the arm (brachial artery), obtained with the use of the hand held Doppler. (2003)The ABI has been validated against angiographically confirmed disease and found to be 95% sensitive and almost 100% specific. (Bernstein and Fronek, 1982) Peripheral arterial disease has been traditionally defined as an ABI < 0.9. However, studies show that abnormally high ABI (>1.3), which occurs from non-compressibility of the foot arteries, is associated with diabetes,(Aboyans et al., 2008) major amputation, (Everhart et al., 1988; Silvestro et al., 2006)cardiovascular and cerebrovascular disease (Alzamora et al., 2013; Allison et al., 2008)as well as mortality(O'Hare et al., 2006; Resnick et al., 2004). Hence, some workers have used a range of <0.9 -1.3 to define normal ABI. (Aboyans et al., 2008)

Numerous studies have determined the burden of PAD amongst patients with diabetes in developed (Marso and Hiatt, 2006; Aboyans et al., 2008) and developing countries.(Umuerri and Obasohan, 2013; Rheeder et al., 2004) In a 2010 review, the prevalence of PAD amongst DM patients was reported as 8% to 33%.(Jude et al., 2010) The wide variation in its prevalence was attributed to differences in the methods used to define PAD.(Hiatt et al., 1995)

In Nigeria (Edo et al., 2013; Adeleye, 2005) and other developing countries (Ahmad et al., 2013; Chalya et al., 2011), most workers have used clinical methods as the only means for assessing PAD in patients with DMFS. In a recent Nigerian study, 27(44.3%) of the 61 patients with DMFS were diagnosed with PAD using reduced/absent lower limb arterial pulsation.(Edo et al., 2013) Other studies in Nigeria have documented the use of ABI <0.9 alone in PAD diagnosis amongst patients with DMFS. (Ikem et al., 2010; Umuerri and Obasohan, 2013; Ogbera et al., 2008)or a combination of clinical methods and ABI <0.9 to define PAD amongst patients with DMFS.(Ekpebegh et al., 2009)There is limited data on the prevalence of PAD defined as ABI <0.9 and >1.3.

We hypothesized that the prevalence of PAD using ABI of < 0.9 or >1.3 amongst patients with DMFS would be higher than previous reports. Our aim was therefore to determine the prevalence of peripheral arterial disease amongst patients with DMFS in a tertiary health care centre using ABI cut off values of <0.9 or >1.3 to define PAD. We also set out to determine the pattern of abnormal ABI amongst our sudy participants.

METHODS

A retrospective study of the hand held Doppler report of patients with DMFS referred to the Diabetes, Endocrinology and Metabolism Unit of the Lagos University Teaching Hospital over a 1 year period was conducted. The Lagos University Teaching Hospital is the largest tertiary hospital in Lagos State, and serves as a referral centre for other peripheral hospitals from within and outside the state.

Approval for the study was obtained from the Health Research and Ethics Committee of the Lagos University Teaching Hospital, Idi- Araba, Lagos State.

Routine Practice

A hand held Doppler (Lifedop 150 Basic by Summit technologies) which used a 8Hz probe was used to measure the systolic blood pressure of the brachial, the dorsalis pedis and posterior tibial arteries.

The procedure was done with the patient in the supine position, having rested for at least 10 minutes. Doppler gel was applied over the probe which was placed at 45^0 to 60^0 to the surface of the skin. The probe was moved around the surface landmark of the artery until the clearest signal was heard. The cuff was then inflated about 20 mmHg above the level at which the signal was no longer heard and subsequently deflated slowly to detect the signal reappearance. The signal disappearance corresponds to the SBP. The brachial pressure on one arm was first determined, followed by the ankle pressures of the dorsalis pedis and posterior tibial arteries bilaterally and then the brachial pressure on the other arm. The higher values of the brachial and ankle pressures were used to calculate the ABI.

Data Collection

The hand held Doppler report was used to retrieve patients' data on demographic variables, history of hypertension, smoking and foot affected by DMFS.

Outcome Variable

Peripheral arterial disease (PAD) was the main outcome variable. It was defined as an ABI of <0.9 or >1.3.

Statistical Analysis

Statistical analysis was done with Epi info 3.5.3 software. Continuous variables were presented as means and standard deviations and categorical variables were expressed as frequencies with accompanying percentages. The differences between the groups was compared using the using the Student's test for continuous variables and chi square test for categorical variables. The odds ratio and the corresponding 95% confidence intervals (CI) were presented. For the chi-square test, where the expected value of a cell was <5, the Fisher's exact test results was used. Statistical significance was set at $\leq p\ 0.05$.

RESULTS

Demographic and Clinical Characteristics

Seventy-three (73) patients' hand held doppler ultrasound reports for DMFS were available for analysis. This comprised of 28 (38.4%) females and 45 (61.6%) males. Their age ranged from 26 to 79 years. Female and male patients with DMFS had similar demographic and clinical characteristics. The demographic and clinical characteristics of the patients are shown in Table 1.

Prevalence of Peripheral Arterial Disease (PAD)

Peripheral arterial disease was present amongst 39(53.4%) of the 72 patients with DMFS as shown in Figure 1.

Amongst the 39 persons with PAD, 11 (28.2%) had ABI >1.3. Figure 2 shows the distribution of abnormal ABI in the study population.

Association of Peripheral Arterial Disease (PAD) with Demographic and Clinical Characteristics

Amongst these patients with DMFS, there was no significant difference in the age, gender distribution, smoking status, laterality or presence of hypertension in patients who had peripheral arterial disease compared to those who did not have PAD (Table 2).

DISCUSSION

Diabetic foot ulcers occur in a background of the triad of infection, neuropathy and peripheral arterial disease. The aim of our study was to determine the prevalence of PAD amongst patients with DMFSs using the criteria of ABI of <0.9 or >1.3, derived from a hand-held Doppler.

In this study, the prevalence of PAD was high (53.4%) and was not significantly associated with age, gender, history of hypertension, smoking or laterality of foot affected by the ulcer. While some studies on DMFS have reported a lower rate of PAD compared to the present study(Ekpebegh et al., 2009; Edo et al., 2013), others have described the contrary(Ikem et al., 2010; Adeleye, 2005; Ahmad et al., 2013) The reason for this can be ascribed to differences in the patients characteristics such as age, sex, presence of other risk factors for PAD, criteria used in determining the presence of PAD as well as the study design(prospective vs retrospective).

Many (Adeleye, 2005; Edo et al., 2013; Nyamu et al., 2003; Otu et al., 2013) studies from Africa except a few (Ekpebegh et al., 2009; Ikem et al., 2010)have used clinical methods as the sole criterion in diagnosing PAD among patients with DMFS. Some of the studies have reported lower prevalence of PAD compared to that found this study (Adeleye, 2005; Edo et al., 2013)varying from 31% to 44.3%. Ekpebegh et al (Ekpebegh et al., 2009) reported a lower prevalence (31%) of PAD in the study of 42 patients with DMFS compared to this study. A combination of reduced/absent pedal pulsations or ABI <0.8 or > 1 was used to diagnose PAD. The difference in PAD prevalence could be explained by the younger age of their patients (56.1 years in the study by Ekpebegh et al vs 62.4 years in this study) and the use of clinical methods in addition to ABI by Ekpebegh et al, which have been shown to underestimate PAD.

On the other hand, Ikem et al,(Ikem et al., 2010) in their study amongst patients with DMFS in Ife, Nigeria, found 31(76.4%) of the 46 patients with diabetic foot ulcers to have ABI<0.9. (Ikem et al., 2010) Although, the age and gender composition for both studies were similar, the difference in rate of PAD can be explained by the higher proportion of other established risk factors for PAD in their patient population. This includes a history of tobacco use which was observed in 26.1% of the patients in their study and comparatively higher than the present study (13.8%). Furthermore, 73.9% of the DMFS patients in the Ife study had hypertension, which is much higher than ours (49.2%). Other risk factors for PAD that have been identified in DM patients include hypertension, (Carbayo et al., 2007; Adler et al., 2002; Umuerri and Obasohan, 2013)smoking, (Carbayo et al., 2007; Adler et al., 2002)older age,(Adler et al., 2002; Marso and Hiatt, 2006; Umuerri and Obasohan, 2013) male gender, (Dormandy and Rutherford, 2000) and hypercholesterolaemia(Carbayo et al., 2007; Adler et al., 2002), duration of diabetes(Adler et al., 2002; Marso and Hiatt, 2006; Umuerri and Obasohan, 2013) and glycaemic control(Adler et al., 2002; Marso and Hiatt, 2006).

In this study, 11(28.2%) patients had an ABI > 1.3, indicative of medial arterial calcification. Although occlusive vascular disease cannot be assessed when arterial are poorly compressible, it has been demonstrated that with the use of alternative tests such as the toe-brachial index, 62.2% to 80% of such patients would have occlusive peripheral arterial disease.(Suominen et al., 2008; Aboyans et al., 2008) Thus, some workers have proposed that ABI>1.3 should be considered PAD-equivalent amongst persons with diabetes. (Aboyans et al., 2008) An ABI >1.3 has been associated with excess mortality, cerebrovascular disease(Alzamora et al., 2013; Allison et al., 2008), heart failure, (Allison et al., 2008)and lower quality of life(Allison et al., 2008). It is

noteworthy that a significant proportion of our study participants who had high ABI, and consequently increased risk of cardiovascular events
and mortality independent of other cardiovascular risk factors would have been missed if ABI <0.9 was solely used to diagnose PAD.
The strength of this study was the use of an objective means to assess peripheral arterial disease and the relatively larger sample size compared to previous studies. Our limitations includes the possibility that not all patients in our institution with DMFS during the study period were assessed with the hand-held Doppler.

CONCLUSION

The burden of PAD using the hand held Doppler to calculate ABI amongst these patients with DMFS was high. Over a quarter of these patients had an abnormally high ABI which impacts negatively on cardiovascular risk and ulcer outcome. Such simple devices should be made more readily available, at least in major hospitals in resource poor settings for better management of patients with DMFS.

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TABLE & FIGURE CAPTIONS

Table 1: Demographic and clinical characteristics of the study population

Variable	**Female n=28 (%)**	**Male n=45 (%)**	**Total n (%)**
Age (years)			
Mean (SD)	60.5 (11.6)	63.5 (10.7)	62.4 (11.1)
Hypertension			
Yes	8(27.6)	21(72.4)	29(39.7)
No	12(40.0)	18(60.0)	30(41.1)
Not indicated	8(57.1)	6(42.9)	14(19.2)
Smoking			
Yes	0(0.0)	8(100.0)	8(11.0)
No	21(42.0)	29(58.0)	50(68.5)
Not indicated	7(46.7)	8(53.3)	15(20.5)
Laterality			
Right	5(23.8)	16(76.2)	21(28.8)
Left	11(55.0)	9(45.0)	20(27.4)
Bilateral	3(50.0)	3(50.0)	6(8.2)
Not indicated	9(34.6)	17(65.4)	26(35.6)

Table 2- Association of peripheral arterial disease with demographic and clinical characteristics

Variable	**PAD Present n=39**	**PAD Absent n=34**	**P value**
Age (years)	64.2(9.6)	60.3(12.3)	0.143
Gender			
Females	17(60.7)	11(39.3)	0.457
Males	22(48.9)	23(51.1)	
Hypertension [a]			
Yes	14(48.3)	15(51.7)	0.898
No	15(50.0)	15(50.0)	
Smoking [a]			
Yes	4(50.0)	4(50.0)	0.607
No	23(46.0)	27(54.0)	
Laterality [a]			
Right	11(52.4)	10(47.6)	0.781
Left	9(50.0)	11(50.0)	
Bilateral	2(33.3)	4(66.7)	

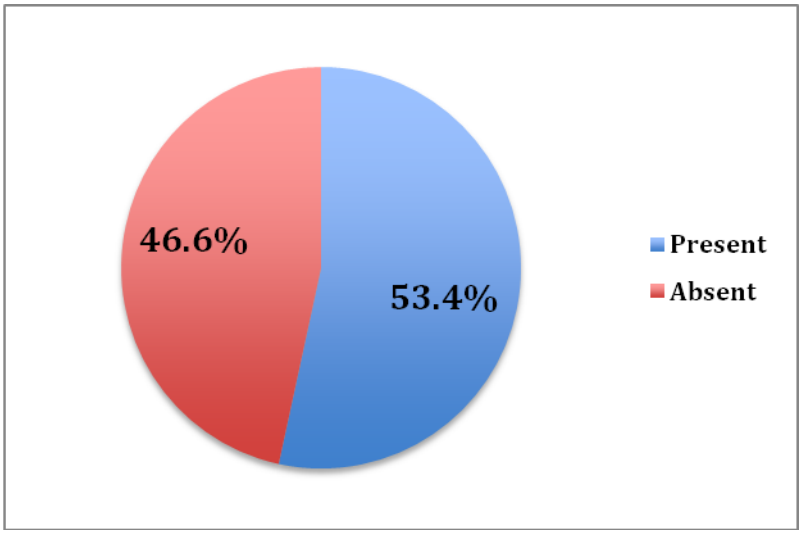

Figure 1- Prevalence of peripheral arterial disease in the study population

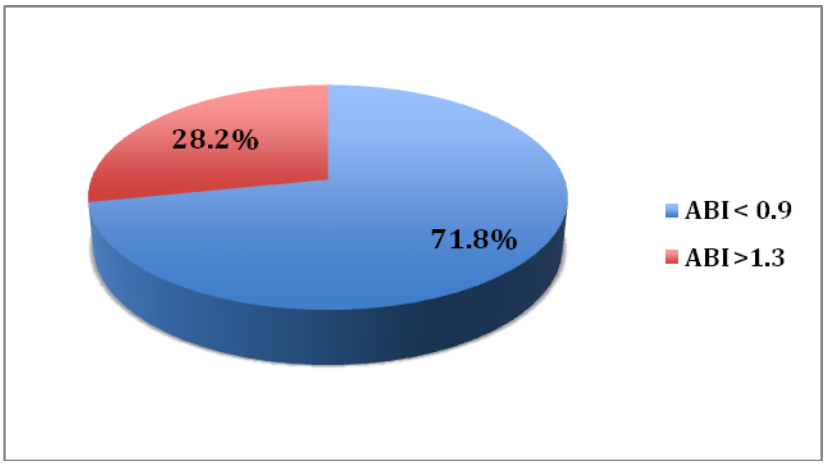

Figure 2- Pattern of abnormal Ankle Brachial index amongst patients with PAD

PROSTATE SPECIFIC ANTIGEN: REFERENCE INTERVAL IN HEALTHY MALES 40 YEARS AND ABOVE IN LAGOS, NIGERIA USING ENZYME LINKED IMMUNOSORBENT ASSAY METHOD

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ABSTRACT

Background: Prostate cancer is the commonest type of cancer in men aged 40 yrs and above. Serum Prostate Specific Antigen (PSA) is a widely used analytical test for screening, detection, staging and monitoring of response to treatment of prostate cancer. However, there is considerable overlap in values obtained in patients with prostate cancer and those with benign conditions, such as benign prostatic hyperplasia. In addition, most of the PSA test kits in use in Nigeria have reference intervals derived from Caucasians and it has been observed that blacks have higher levels of serum PSA than whites hence making this local study a necessity. **Objective:** The aim of this study is to determine the reference intervals for serum total prostate specific antigen in healthy males of age 40years and above in Lagos, Nigeria. **Methods:** Ethical clearance was obtained from the Lagos University Teaching Hospital Ethics Committee. This study is a prospective study carried out on randomly selected 130 Nigerian men living in Lagos between the ages of 40 and 80 years that met the set inclusion and exclusion criteria who were apparently healthy (as determined by a health assessment questionnaire and vital signs) after informed consent was obtained from study volunteers. Blood samples were collected from each subject before 10am for serum total PSA assay. Quantitative determination of PSA in serum was done using an enzyme linked immunosorbent assay (ELISA) kit and read out using Sirio S microtitre well reader (SEAC, Italy). PSA Reference interval was derived using the non-parametric percentiles. **Results:** The reference interval for serum total PSA is 0-4.6 ng/ml. There was a weak but significant positive correlation between serum total PSA and age of subjects ($r = 0.24$, $p < 0.01$). **Conclusion:** The PSA reference interval derived from this study was different from that of most PSA test kits but the difference was not clinically significant.

Keywords: *Prostate Specific Antigen, Prostate cancer, reference interval, ELISA, benign prostatic hyperplasia.*

INTRODUCTION

Prostate Specific Antigen (PSA) is one of the most promising and clinically useful tumour markers for prostate cancer available currently and it is one of the few organ specific tumour markers[1]. Serum PSA is a widely used analytical test for screening, detection, staging and monitoring of response to treatment of prostate cancer. The role of PSA in prostate cancer has been reviewed [2,3,4,5]. Prostate Cancer is one of the leading cancers in men aged 40 yr and above and when detected early, it is potentially curable. Its prevalence is said to be on the increase in Nigeria[6]. Early screening for prostate cancer includes early testing for serum prostate specific antigen (PSA) and it has been observed that blacks have higher levels of prostate specific antigen than whites [7,8]. In addition, because PSA is only organ specific and not necessarily tumour specific, there is considerable overlap in values obtained in patients with prostate cancer and those with benign conditions, such as benign prostatic hyperplasia and prostatitis. In an effort

towards improving the ability of PSA testing to detect early prostate cancer, several approaches have been proposed. These includes the use of digital rectal examination, PSA density (defined as the ratio of serum PSA concentration to prostatic volume as determined by transrectal ultrasonography), Age –Specific Reference Ranges, PSA velocity (change in PSA concentration with time), measuring free PSA, complexed PSA, or the ratio of these forms to total PSA for early detection of prostate cancer [2,3,4,5].

The detection of prostate cancer using serum PSA is improved when age specific reference ranges are used, but these ranges currently in use in Nigeria have been derived from white (Caucasian) populations [9]. Age specific reference ranges are however different in White, African American, Asian and African populations [8-13]. If the current PSA age specific reference intervals obtained in Caucasians were used in screening black men, with Specificity kept at 95 %, 41 % of cases of prostate cancer would be missed in black men [8]. The Nigerian population specific reference intervals of serum PSA must be defined in order to improve the sensitivity and specificity of the test and reduce the number of prostate biopsies in the Nigerian population. A published serum PSA reference interval study found in literature was carried out using Radioimmunoassay at the University College Hospital, Ibadan in 1997 [13]. This present study carried out among black African population uses a cheaper, more user and environment friendly enzyme immunoassay technique; it is thus justified given the present reports of variations in serum prostate specific antigen with age and race [8-13].

METHODS

Ethical clearance was obtained from the Lagos University Teaching Hospital Ethics Committee. This study is a prospective study carried out on randomly selected 130 Nigerian men living in Lagos between the ages of 40 and 80 years that met the set inclusion and exclusion criteria who were apparently healthy (as determined by a health assessment questionnaire and vital signs) based on the guidelines and recommendations of the International Federation for Clinical Chemistry (IFCC) for the determination of reference intervals[14-19]. Both parents of the study subjects must be Nigerians to qualify for the study. Informed consent was obtained from the volunteers for the study before each study subjects were recruited. Excluded from the study were - males less than 40 years of age, males that have undergone prostatectomy, males with symptoms and signs of acute or chronic urinary retention, prostatitis, benign prostate hyperplasia or carcinoma of the prostate. Also excluded were patients who have had Digital Rectal Examination, Transrectal Ultrasound or Transurethral biopsy of the prostate within the last 24 - 72 hours preceding collection of samples for PSA assay. Structured Questionnaires were completed by all subjects in addition to history taking and clinical examination before sample collection. The age, weight, height of each subject was measured. About 5ml of venous blood was collected from each subject into plain non-anticoagulated tubes. The serum was separated and stored at -20˚C till time of analysis which were done in batches. Repeated freezing and thawing of samples were avoided. Haemolysed, icteric and lipaemic samples were excluded from the study.

An enzyme linked immunosorbent assay (ELISA) kit (DIAGNOSTICS AUTOMATION INC,USA) for total PSA was used for quantitative determination of PSA in serum and read out using a Sirio S microtitre well reader (SEAC, Italy). The PSA ELISA test is a solid phase two-site immunoassay employing the use of anti-PSA monoclonal antibody labeled with horseradish peroxidase is used as the tracer. The serum PSA data followed a non-gaussian pattern hence the use of non-parametric statistical methods to calculate the reference intervals using the 2.5 and 97.5 percentiles. Least squares regression models and correlation models were constructed with

the data to evaluate the association between serum PSA levels, age and other demographic data. Data entry and analysis was performed using SPSS version 11.0 package.

RESULTS

The distribution of serum total PSA in the population was non-Gaussian hence the use of non-parametric percentile method as recommended by the IFCC for determination of the reference intervals. The reference interval for total PSA is 0 – 4.6 ng/ml (Confidence Interval; 0 – 0, 3.2 – 6.0 ng/ml) (See Table 1)

The PSA values increased gradually with increasing age with the elderly (60 – 71 years) having the highest level of serum total PSA.

There was no statistically significant difference between the mean total PSA of age group 40 – 49yr and 50 – 59yr ($p>0.05$). There was a statistically significant difference between the mean total serum PSA in these various groups combined using the Kruskal-Wallis Test ($p< 0.05$).

There is a significant association ($p <0.01$) between increasing age in years and PSA concentrations. There was a significant ($p<0.01$) correlation between serum Total PSA and Age of subjects ($r = 0.24$). Intra-assay coefficient of variation of PSA assay was 23% while the inter-assay coefficient of variation was 21.3% at normal PSA concentration.

DISCUSSION

In this study, we investigated the levels of serum PSA healthy men aged 40years and above with the aim of determining the reference interval of serum total PSA and found out that the PSA reference interval derived from this study was different from the 0-4ng/ml of the current IFCC recommended Caucasian derived reference interval used by most PSA test kits even though the difference is not clinically significant. Racial differences in serum PSA values have been demonstrated [8-13]. Vollmer demonstrated that for the same volume of prostate gland, blacks produce more PSA than whites [7]. Ted *et al* also reported that PSA concentration in black men both with and without clinical evidence of prostate cancer are significantly higher than those in similar white men [8]. Morgan *et al* observed that same increase in serum PSA levels in blacks relative to whites [24]. No satisfactory explanation has been advanced for this observation, however, possible mechanism include higher levels of testosterone in young black men as compared with a similar group of whites which suggest higher levels of stimulation by androgens leading to more production of PSA in blacks [8]. Other possible mechanism that have been proposed are higher cell turnover in both benign and malignant prostate tissues of blacks with subsequent release of PSA and greater tumor-vascular interface in blacks which might leads to more efficient transfer of PSA to the bloodstream [7,8.]

A previous Nigerian study reported a non-Gaussian distribution of serum PSA; this was also demonstrated in this present study [13]. Ted *et al* also reported the same log normal distribution of serum PSA in both Caucasian and black populations [8]. In the Abbiyesuku *et al* study, no subject below the age of 50 years had serum PSA value greater than 2.0 ng/ml which is different from that of this present study in which four subjects below the age of fifty years had PSA values greater than 2.0 ng/ml. This may be as a result of the spread of our age range (40-80years) compared to the one used by Abbiyesuku *et al* which included young adults of up to 22years of age and also the difference in analytical methodology used (i.e. Radioimmunoassay versus Enzyme immunoassay).

The present study showed a significant rise in PSA values with advancing age. Similar findings have been reported among other races [11, 20, 21]. This age related increase in PSA values with advancing age has been shown to be related to prostate gland volume which increases with

advancing age [20, 22, 23]. This might explain the increasing age specific reference interval derived for the different age group of this current study.

From this study, it has been possible to determine the reference interval for serum total PSA in healthy males of 40years and above in Lagos including age and weight specific reference intervals.

LIMITATIONS: includes high non- compliance rate from subjects approached to consent for the study and high cost of reagent for PSA analysis. Another limitation of this study is the unwillingness of most subjects to consent to digital rectal examination and prostate biopsy which would have strengthened the study by ascertaining that recruited study subjects classified by the health assessment questionnaire and vital signs as having an healthy prostate gland have PSA levels reflecting the true state of health of the prostate gland.

ACKNOWLEDGEMENTS

I thank the almighty God for helping me through this project. My deepest appreciation goes to my mentors Professor O.A. Afonja and Dr. (Mrs.) O.O. Oladipo. I also appreciate Dr. Erinomo, Dr. Awelimobor, Dr. Sobowale and Dr. Ayantuga for helping me with my data collection within and outside LUTH.

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TABLE & FIGURE CAPTION:

Table 1: Serum total PSA age group specific Reference Interval

	Age group 40-49yr	**Age group 50-59yr**	**Age group 60-71year**	**Age group combined 40 – 71years**
Sample size (n)	60	53	17	130
Mean PSA (ng/ml)	0.82	1.03	1.57	1.01
Percentiles 2.5^{th}	0.0	0.0	0.0	0.0
97.5^{th}	3.4	3.9	6.0	4.6

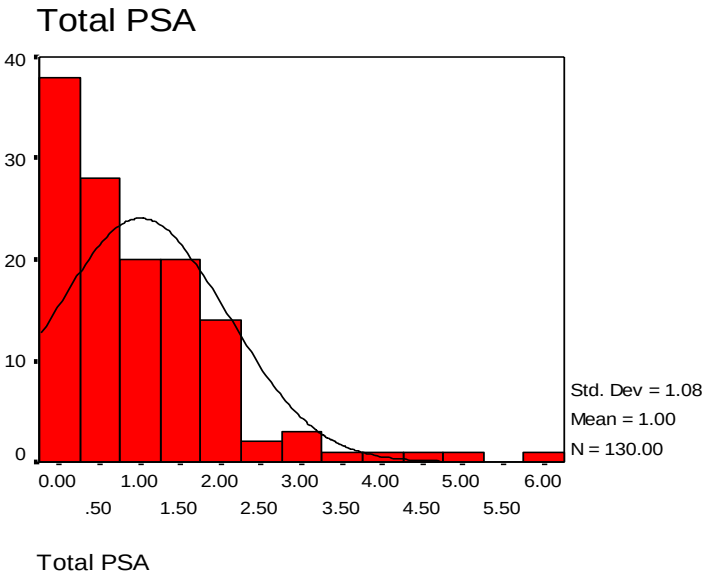

Figure A: Frequency Histogram of serum total PSA distribution irrespective of weight of subjects

THYROID FUNCTION IN ADULT NIGERIANS WITH METABOLIC SYNDROME

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ABSTRACT
Background: Metabolic syndrome and thyroid dysfunction are two common disorders encountered in the metabolic clinic. Recently, there has been increased interest in the association between the two disorders because of the similarities between symptoms of hypothyroidism and components of the metabolic syndrome. While some reports suggest that metabolic syndrome is associated with subclinical hypothyroidism, this concept is largely under investigated in Nigerian adults with metabolic syndrome. **Objectives:** The aim of this study is to determine the thyroid function status of adult Nigerians with metabolic syndrome and determine the association, if any, between metabolic syndrome and thyroid function. **Methods:** This was a cross sectional study of one hundred and fifty adults, members of staff of the College of Medicine of the University of Lagos. The participants were recruited using a cluster random sampling method. The Ethical Research & Review Committee of the institution approved the study protocol and signed informed consent was obtained from the participants. The statistics was analysed using the IBM SPSS Software of version 19.0. The Student's t test, Chi square test and multivariate regression analysis were employed for the analysis. Statistical significance was set at $p < 0.05$. **Results:** Thirty nine (twenty-six percent) of the study participants had metabolic syndrome and one hundred and eleven (seventy-four percent) of the study participants did not have metabolic syndrome, served as controls. Those who had metabolic syndrome group were significantly older (p=0.03), metabolic syndrome was significantly associated with the female gender (p=0.0002), higher systolic blood pressure (p=0.0034), diastolic blood pressure (p=0.0009), waist circumference (p<0.0001), body mass index (p<0.0001), waist-hip ratio (p=0.003), fasting serum glucose (p=0.0457) and free thyroxine (fT4) levels (p=0.0496). Those with metabolic syndrome had significantly lower HDL (P=0.004) and free triiodothyronine (fT3) levels (p=0.037).There was no statistically significant difference in the thyroid stimulating hormone (TSH) levels between individuals with and without metabolic syndrome. Thirty–three percent of the metabolic syndrome cases had sick euthyroid syndrome (p= <0.0001). In multivariate regression, waist circumference was significantly and inversely associated with the sick euthyroid syndrome (p=0.011). **Conclusion:** Metabolic syndrome is associated with the sick euthyroid syndrome in adult Nigerians.

Keywords: *Thyroid, Metabolic Syndrome; Hormone; Disorder, Diastolic blood pressure*

INTRODUCTION

The metabolic syndrome is associated with increased life-time risk for atherosclerotic cardiovascular disease [1]. The prevalence of metabolic syndrome has increased greatly not only in industrialized nations [2] but in developing countries as well [3, 4]. Metabolic syndrome and thyroid dysfunction are encountered commonly in the metabolic and endocrinology clinics, often times occurring together [5, 6]. Some studies have published a high prevalence of thyroid disorders in individuals with metabolic syndrome [5, 6]. Recently there has been increased interest in the association between thyroid function and metabolic syndrome based on the notion that triiodothyronine controls metabolic and energy homeostasis and influences body weight, thermogenesis, lipolysis and metabolism of cholesterol [7].

The criteria defining metabolic syndrome according to the National Cholesterol Education Programme-Adult Treatment Panel III (NCEP-ATPIII) [8], require a combination of at least 3 of the following 5 criteria: Abdominal circumference ≥ 102 cm in males or ≥ 88 cm in females, HDL cholesterol < 1.03 mmol/L (< 40 mg/dL) [males] or < 1.3 mmol/L (< 50 mg/dL) [females], triglycerides ≥ 1.7 mmol/L (≥ 150 mg/dL), blood pressure ≥ 130/85 mmHg or the patient receiving hypotensive treatment and fasting glycaemia > 6.1 mmol/L (> 110 mg/dL).

This cluster of metabolic abnormalities is associated with increased risk for atherosclerotic cardiovascular disease and type 2 diabetes mellitus [9].

In hypothyroidism, there is increase in low density lipoprotein cholesterol (LDL), total cholesterol (TC) triglyceride (TG) and weight gain which may lead to obesity, and an increase in blood pressure [10]. These similarities in symptoms of obesity, dyslipidaemia and dysglycemia in both disorders have lead researchers to posit that metabolic syndrome may be a consequence of some occult abnormality of the thyroid gland [11, 12]. Other studies have also pointed to thyroid disorders as being complications of the metabolic syndrome and type 2 diabetes [13, 14].

Many researchers have documented an association between components of the metabolic syndrome and serum levels of TSH, fT4 and fT3 [15-18]. Many studies have associated subclinical hypothyroidism with metabolic syndrome [5, 6, 11]. Other studies have found the same associations in the older age group [19, 20]. There is paucity of data on thyroid function in metabolic syndrome in adult Nigerians. The aim of this study therefore is to determine the association of thyroid disorder with metabolic syndrome in adult Nigerians.

METHODS

Study Design

This was a cross sectional study of one hundred and fifty adults, males and females, members of staff of the College of Medicine of the University of Lagos. The participants were recruited using a cluster random sampling method. The units in the university were grouped into clusters. The cluster to be studied was selected by simple random sampling method. Every individual within the cluster who met the selection criteria, and who gave consent, was recruited for the study. The Ethical Research & Review Committee of the institution approved the study protocol and informed consent was signed and obtained from the participants.

Inclusion and Exclusion Criteria

The inclusion criterion was adult males and females between 35 and 70 years of age. Adults with overt thyroid disease, on steroid medication and known diabetics were excluded from the study. Pregnant women were also excluded from the study.

Individuals who met the above criteria and who agreed to participate in the study, reported on the morning of the study after an overnight (10-12 hours) fast. 5mls of venous blood was collected from the ante cubital vein.

Anthropometric measurements were taken. Abdominal obesity was determined by measurement of the waist circumference in centimetres, using the pubic crests and the umbilicus as land marks.The hip circumference was measured from the farthest point on the gluteus using the anatomical neck of the femur as land marks. The blood pressure was determined using the Accoson's Mercury Sphygmomanometer (cuff size 15×43cm). The subjects were seated and rested for 30minutes before measurement. The systolic blood pressure was taken at the first korotkoff sound and diastolic at the fifth korotkoff sound. The average of two readings taken fifteen minutes apart was used.

The total, LDL, HDL cholesterol, triglyceride and glucose concentrations were determined on fasting serum samples[21] using reagents from Randox Laboratories Limited, Antrim, UK, BT 29 4QY, on semiautomatic biochemistry analyser BS3000P-Sinnowa Medical Science and Technology company limited, Nanjing, China (211135). Free T3, T4 and TSH concentrations

were determined using reagents from Inteco Diagnostics, UK, E8 3DY, by an enzyme linked immunoassay technique [22] on Acurex Plate Read - Acurex Diagnostics, Ohio, USA (419-872-4775)

Statistical analysis

The data was analysed using the IBM SPSS Software of version 19.0. The Student's t test was used to test the differences in the mean values for the continous variables. Chi square test was used to test the differences in proportion of the catergorical variables. Multivariate regression analysis was also employed to test for strength of relationship between continuous variables. Statistical significance was set at $p < 0.05$.

RESULTS

Table 1 shows the clinical and laboratory characteristics of subjects with and without metabolic syndrome.

The associations remained significant even after adjustments for age and sex. Table 1 shows the adjusted and unadjusted p values.

Table 2 shows the thyroid function status of the study participants

The sick euthyroid syndrome was significantly associated with metabolic syndrome while most of the controls were euthyroid.

Table 3 shows the multivariate regression of components of the metabolic syndrome on sick euthyroid syndrome.

Only waist circumference showed an association with the sick euthyroid syndrome.

DISCUSSION

The study identified sick euthyroid syndrome as the commonest abnormality of the thyroid in adult Nigerians with metabolic syndrome. Low fT3 levels which is the most consistent finding in sick euthyroid syndrome was significantly associated with the metabolic syndrome and had mean values significantly lower in the metabolic syndrome. The ability of the study to bring out these findings highlights its strength. The study contributes to medical practise as awareness of these alterations will help in avoiding errors in diagnosis of thyroid disorders and inappropriate therapy. The limitations of the study include unavailability of electronic health records/poor record keeping in the communities which would enable access to data from a larger section of the population at significantly lower cost.

This study showed a statistically significant difference in the components of the metabolic syndrome between the study group with metabolic syndrome and the control group, in keeping with findings in literature [2, 3] and these associations remained after adjustments for age and sex (Table 1).

From this study, the sick euthyroid syndrome is the most common abnormality of the thyroid gland in people with metabolic syndrome occurring in 33.3% of the group with metabolic syndrome compared to 1.8% in the control group ($p<0.0001$). This differs from findings by some authors working in different regions and with different races. Workers in India [5, 16], Taiwan [19] and Korea [18], found a high prevalence of sub clinical hypothyroidism in people with metabolic syndrome. Heima et al [23] working in Amsterdam also found an association between higher TSH levels and metabolic syndrome in euthyroid subjects. Iodine deficiency, autoimmune thyroiditis and mutations in the TSH receptor genes are some of the hypothesis put forward to explain the association between increasing TSH, obesity and subclinical hypothyrodism in these populations [7] Longitudinal studies are required to determine whether the metabolic alterations are a cause or a consequence of the thyroid dysfunction. In these populations, a high prevalence

of metabolic syndrome was also found in patients with subclinical hypothyroidism [11]. In subclinical hypothyroidism, there is altered thyroid function with normal feedback regulation (fT4 at the lower limit of normal range and increased TSH also within normal range) was thought to be the primary event that induces alterations in energy expenditure with subsequent increases in BMI and weight and other cardio metabolic risk factors [24, 25]. Studies have also recorded high prevalence rates for both subclinical hypothyroidism [26] and metabolic syndrome [5] in these regions and these may further explain the associations.

The differences in thyroid function in metabolic syndrome observed in this study may also be explained by genetic differences across regions as studies on the genetic causes of the metabolic syndrome have demonstrated no single locus reproducibly linked with the metabolic syndrome across populations, partly explained by the effect of ethinicity and by the complexity of the metabolic syndrome itself [27].
Ethnic and regional differences in reference values for TSH which appear to be lower in the Nigerian population compared to India, Europe and USA [26, 28], may also contribute to the differences in thyroid function in the metabolic syndrome.
In the present study, fT3 was significantly lower and fT4 significantly higher in the group with metabolic syndrome with no significant difference in the TSH values in both groups (Table 1), this is in keeping with the sick euthyroid syndrome. In the sick euthyroid syndrome, the most consistent finding is a low or low normal fT3 value with raised reverse T3. Also seen, is a low normal or normal TSH and fT4 is normal or mildly elevated [10].

The sick euthyroid syndrome is an abnormality of thyroid hormone concentration seen in a wide variety of Non thyroidal illnesses. The pathogenesis of the disorder is associated with inhibition of the hepatic enzyme, 5^1 monodeiodinases which catalyzes the conversion of fT4 to active fT3 with increase in reverse T3 [10]. These changes may also be mediated in part by inflammatory cytokines acting at the level of the hypothalamus and pituitary [29]. It is not clear whether it is an adaptive response which lowers tissue energy requirement during systemic illness or a maladaptive response leading to damaging tissue hypothyroidism [29]. The thyroid abnormalities normalize as the patient recovers from illness [10]. Treatment of obesity with hypocaloric diet also causes changes in thyroid function resembling the sick euthyroid syndrome [30], although the effect of diet therapy was not assessed in the present study. Longitudinal studies looking at the effect of diet therapy and long term effects of low fT3 values in these individuals are needed. It is possible that this tissue hypothyroidism could set off a sequence of metabolic dysregulations to worsen the individual's metabolic condition.

In this study, employing multivariate regression, central obesity determined by the waist circumference was associated with the sick euthyroid syndrome (Table 3). Some studies also found associations between thyroid dysfunction and obesity in the metabolic syndrome [7, 31]. Obesity appears to be the link between metabolic syndrome and the sick euthyroid syndrome.
Fat cells produce leptin and are thus considered an active endocrine organ [24]. In addition to the role of leptin in regulating energy homeostasis, [24] leptin is also an important neuroendocrine regulator of the hypothalamic-pituitary-thyroid axis [32] by regulation of TRH gene expression in the paraventricular nucleus. Leptin also affects thyroid deiodinase activities with activation of T_4 to T_3 conversion [24, 33]. Available data support the concept of an inverse relationship between thyroid hormone and leptin [24, 32-33] and would explain the inverse relationship between waist circumference and sick euthyroid syndrome found in this study (Table 3)

CONCLUSION

Metabolic syndrome is associated with changes in thyroid function, in adult Nigerians, consistent

with the sick euthyroid syndrome and abdominal obesity appears to be the direct cause. At present, the patients with sick euthyroid syndrome are considered to be essentially euthyroid and thyroid replacement therapy is not administered. Awareness of these alterations helps in avoiding errors in diagnosis of thyroid disorders and inappropriate therapy**.** Further studies are however needed to examine the long term effects of low fT3 on the metabolic status of individuals with metabolic syndrome.

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Table 1: Clinical and laboratory characteristics of subjects with and without metabolic syndrome

Characteristics	**Metabolic syndrome N=39/(%) Mean±SD**	**No metabolic syndrome N=111/(%) Mean±SD**	**P value Unadjusted, adjusted**
Gender Females males	 31(79.5) 8(20.5)	 50(45) 61(54.9)	 0.0002*
Age(years)	49.61±7.84	46.33±8.10	0.030*
SBP(mmHg)	131.97±17.54	122.42±17.14	0.003*, 0.028*
DBP(mmHg)	83.10±11.25	75.77±11.78	0.0009*, 0.0013*
WC(cm)	99.61±9.11	89.77±9.37	<0.0001*, 0.017*
BMI(kg/m^2)	30.60±4.41	26.37±5.93	<0.0001*, 0.011*
WHR	0.88±0.05	0.85±0.05	0.003*, 0.017*
Glucose(mmoles/L)	5.24±2.07	4.61±1.54	0.045*, 0.049*
TG(mmoles/L)	1.90±0.13	1.84±0.20	0.091, 0.120
HDL(mmoles/L)	1.26±0.12	1.33±0.14	0.004*, 0.034*
TC(mmoles/L)	5.02±0.44	5.16±0.42	0.08, 0.980
LDL(mmoles/L)	2.90±0.42	2.99±0.45	0.26, 0.441
T_3(pmole/L)	2.86±1.24	3.92±3.09	0.037*, 0.042*
T_4(pmole/L)	17.24±9.94	14.07±8.05	0.049*, 0.039*
TSH(μiU/ml)	1.06±0.88	1.10±0.88	0.794, 0.379

***statistically significant**

Table 2: Thyroid function in the study participants

Thyroid state	**Metabolic syndrome N=39(%)**	**No metabolic syndrome. N= 111(%)**	**P value**
Sick euthyroid syndrome	13(33.3)	2(1.8)	<0.0001*
Euthyroidism	23(58.9)	88(79.2)	<0.0001*
Primary hyperthyroidism	1(2.6)	2(1.8)	0.986
Secondary hypothyroidism	1(2.6)	4(3.6)	0.973
Subclinical hyperthyroidism	1(2.6)	5(4.5)	0.856
Subclinical hypothyroidism	0(0)	0(0)	

Table 3: Multivariate regression of components of the metabolic syndrome on sick euthyroid syndrome

Components of the Metabolic syndrome	**Regression coefficient**	**P value**
SBP(mmHg)	0.75	0.208
DBP(mmHg)	0.92	0.126
WC(cm)	-0.060	0.011*
Glucose(mmoles/L)	0.074	0.613
TG(mmoles/L)	-1.14	0.375
HDL(mmoles/L)	1.596	0.341

*statistically significant

CLIENT SATISFACTION AND QUALITY OF FAMILY PLANNING SERVICES: A COMPARATIVE STUDY OF PUBLIC AND PRIVATE FACILITIES IN LAGOS, NIGERIA

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ABSTRACT
Background: Client satisfaction and quality of care of family services affect contraceptive uptake and continued usage of method. **Aim:** To determine and compare client satisfaction with quality of family planning services between public and private health facilities in an urban area of Lagos, Nigeria. **Methodology:** A cross sectional study was carried out among consecutively recruited 240 women accessing family planning services in July 2013 at public and private health facilities. Data collection was done with exit interview, Client-provider interaction and facility audit questionnaires from MEASURE EVALUATION and analyzed with Epi-Info at 5% significance level. **Results: (public vs private)** mean age was 35.5(5.5) years vs 37.9(7.5) years; mean waiting time (minutes), 24.8(11.7) vs 48.7(17.8). Statistically significant differences were observed in waiting time, (12.5%, 15/120) vs (30%, 36/120) ($p<0.001$); active participation, (95.8%, 115/120) and (100%, 120/120) ($p=0.020$); client received method of choice, (66.3%, 56/80) vs (72.3, 60/83) ($p=0.010$). Overall, private provider clients were better satisfied with services (93%, 112/120) than those in the public facility (88%, 105/120) ($p <0.001$). **Conclusion:** The private provider clients were better satisfied with services. Training of public providers on interpersonal relationship, counseling and communication skills is recommended. Private providers should implement strategies to reduce waiting time.

Keywords: *Client satisfaction, Quality of care, Family Planning, Nigeria*

INTRODUCTION

The level of satisfaction that clients experience having used a service is client satisfaction. It is the differences between the expected service and the experience of the service from the point of view of the client's. Across the globe, understanding and measuring client's satisfaction has become a vital part of hospital/clinic management strategies. Moreover, in most countries quality assurance and accreditation process requires a regular measurement of clients' satisfaction.[1]

A study done to assess the interpersonal and organization dimension of clients' satisfaction revealed that satisfaction influences whether a person seeks medical advice, complies with treatment and maintains a continuing relationship with practitioners.[2] A leading theorist in the area of quality assurance has emphasized that Client satisfaction is of fundamental importance as a measure of the quality of care because it gives information on the provider's success at meeting those client values and expectations, which are matters on which the client is the ultimate authority.[3]

Quality of care is a key component of health care delivery and therefore has an important bearing on client satisfaction. Client satisfaction is a major factor that will determine whether a client will seek medical care and also adhere to a prescribed treatment. Dissatisfaction with health services may result in patients/clients not adhering to treatment regimens and follow up appointments. They may even spread negative information based on their perception to discourage people from using a health service.[4]

Several factors such as low level of knowledge, low quality of services including non-availability of contraceptive commodities, poor attitude of service providers, and low status of women are reported responsible for low utilization of Family Planning services in Nigeria.[5] Hence, the need for, continuous monitoring of quality of care should be based on clients' satisfaction and perception of quality of care.[6]

Studies have shown that one principal determinant of uptake and continued utilization of family planning services is overall client satisfaction with those services.[7,8,9,10] Studies of contraceptive discontinuation rates have indicated that - with the exception of the desire to become pregnant - the principal reason for discontinuation is dissatisfaction with the quality of services.[11]

The total fertility rate in Nigeria is 5.7 and on the average, urban women have 4.9 children. The fact that contraceptive prevalence in Nigeria is still low from 6% in 1990 to 13% in 2003, 14.6% in 2008, so also is the use of modern contraceptive methods; from 4% in 1990, 8% in 2003 to 10% in 2008,[12] thus the quality of care and level of satisfaction may help women who want to avoid pregnancy but who feel uncertain about contraception.[7]

This study was conducted to determine and compare client satisfaction and quality of family planning services between a public and private health facility with large clientele base in Lagos, Nigeria.

METHODS

Study Sites and Setting

The study was conducted in Oshodi-Isolo LGA, Lagos State, Nigeria. At the 2006 Census it had a population of 621,509 people, and an area of 45 square kilometers.[13] Existing public health facilities in the LGA include a general hospital, i.e. the Isolo General Hospital and 12 Primary Health care Centers.[14] In addition, some Non-Governmental Organizations, agencies, and charity groups also established health facilities providing varying degrees of health services. The two health facilities studied were Isolo General Hospital (public) and Planned Parenthood Federation of Nigeria Clinic. Both were purposively chosen because they serve majority of the family planning clients in Oshodi-Isolo LGA, thereby serving as a large pool of desired respondents. The public provider provides family planning services to about 350 clients monthly. The private provider is a private not-for-profit organization that offers family planning services to about 450 clients monthly.

A cross-sectional, comparative study was carried out among women accessing family planning services in July 2013 at these two centers. Only women who came solely for family planning services were included, those who came for other maternity services like ante natal care were excluded. The respondents were interviewed consecutively until the sample size was achieved.

Using the formula for comparing two independent groups, an initial minimum sample size of 94 respondents was calculated using the following parameters; statistical power (80%); 95% confidence interval; satisfaction rates of 46.9%(public) and 63.6%(private).[15] However, 120 women were subsequently interviewed in each facility to allow for 10 % non-response rate, hence giving a total sample size of 240.

Data Collection

A structured interviewer-administered exit client questionnaire, facility survey and client-provider interaction observation tools adapted from MEASURE Evaluation QIQ were used for data collection.[16] The Facility Audit was used to determine the readiness of a facility to deliver services; the observation of the Client-Provider Interaction (CPI) provided information about the exchange between the client and the provider from the perspective of a clinician; and the Client Exit Interview was used to collect information about the client's experience at both health facilities. Four research assistants collected data using the facility survey and client exit interview. One hundred and twenty client exit interviews were conducted for each facility,

making a total of two hundred and forty respondents. While an external nurse with training on providing family planning services conducted the client – provider interaction observation on 30 clients at each centers. The interviews were conducted privately and not within ear shot of service providers so as to ensure un-biased responses.

The research instruments were pretested at similar public and private facilities both in an urban area of Lagos. Ten client exit interviews and one client-provider observations were conducted at each facility. Slight adjustments were made before actual study.

Data Management

Data analysis was done with Epi-Info 3.5.1 version software package. Frequencies, percentages and means were calculated. Test of significance using chi square (x^2) and t-tests were done at a significance level of 5 % ($p< 0.05$).

Ethical Considerations

Ethical approval was obtained from the Health Research and Ethics Committee of the Lagos University Teaching Hospital. Permission was also obtained from medical directors of the two selected centers. Respondents gave informed written consent before interview and client confidentiality was ensured as the questionnaires were anonymous.

RESULTS

Socio-Demographic Characteristics Of Respondents

A total of 240 respondents were interviewed for the study, one hundred and twenty from each health facility. Respondents in private facility were older than respondent in public facility with mean age of 35.5(5.5) years (public) and 37.9(7.5) years (private). The difference was statistically significant ($p<0.001$). Respondents in public center were better educated than respondents at private center with 96.7% (116/120) of them with post primary educated compared with 89.2% (107/120) in private center. Almost all the respondents 99% (119/120) in public center were married compared to 93% (112/120) in private center Respondents at both centers have same median number of livining children as 3. The difference observed was statistically significant ($p=0.010$). Although about one third of respondents, 29.2% (35/120) desired to have another child in the future at both centers, however, more respondents 43% (23/120) at public center will like to wait for more than 2 years before having another child compared to 18 % (7/120) of the respondent at private center.(Table 1).

Contraceptive History Of Respondents

The most common methods amongst new clients were implant (41.2%, 14/79) and intra uterine device (IUD) (29.4%, 10/34) at public center while at private center; they are IUD (34.6%, 9/26) and injectables (26.9%, 7/26). For the re-visit clients, common methods were injectables and pills at both centers.

Common methods discussed by providers with Clients at both centers include injectables and IUD. Public Provider discussed more about condoms as a contraceptive with 35% (42/120) of client than private providers who discussed condom with 10.8%(13/120) of client. (Table 2).

Client Satisfaction And Quality Of Family Planning Rendered

The mean waiting time (minutes) in private center 48.7(17.8) was longer than the waiting time in public center 24.8(11.7). More respondents 87.5% (105/120) at public center reported satisfaction with the waiting time than respondents 70% (84/120) in private center. The private facility was rated better in maintenance of privacy and treatment by other staff than the public facility. (Table 3)

Perceived clients' rating of the indicators of quality of family planning services showed that private center was rated better than public center in many aspects like provider discussing STI/AIDS, giving instruction on method use, maintenance of privacy, treatment by other staff and active participation of clients. There were significant difference (p=0.050) in treatment by other staff and client who received their method of choice (p=0.010). (Table 4)
Private center rated better than public in communication skills, information discussed and following clinical procedures for injectables, pelvic examination and IUD insertion. The indicators observed during client-provider interaction showed that providers in private center followed clinical procedure guidelines for injectables, pelvic examination and IUD insertion than those in public center. (Table 5)

DISCUSSION

From this study, clients' rating at private center was better than those at public center in most of the perceived measurable indicators such as client participation in selection of method, treatment by other clinic staff, and provider mentioning STI/AIDS during counseling. However, the public center was better with waiting time than private center.
Private respondents were older than public respondents with mean age of 37.9(7.5) years (private) and 35.5(5.5) years (public). Most women would have been married at this age and thus uptake of FP is expected to be higher. The current marital status of respondents in both facilities was in keeping with the reports of 2008 NDHS in which about 70% of women were either formally married or are living together.[17]
Choice and continuous usage of contraceptives may be influenced by clients' family size and fertility intentions. The median number of children reported was 3 for both centers, however one third of the respondent in both centers 29.2% (35/120) desired to have more children in future, this is in keeping with a study conducted in urban health facility where 29% of the FP clients desired to have more children in future.[17,18] This could infer that they came primarily for spacing.

In line with previous studies, about a quarter of FP clients were new.[10] However, the contraceptive methods that clients received differed substantially between new and re-visit clients in both centers. The most frequently used methods were Implants and IUD (for new clients) and injectables (for revisit clients). It is a general belief that the long usage of injectables can cause delay in conception,[19] hence its popularity with re-visit clients who may have completed their family size. This finding however, differs from earlier study carried out in Bangladesh which reported pills as the most commonly used method, followed by IUD and injectables.[20] This may infer that the pill is not popular among our respondents.

One of the important factors related to client perceptions is the waiting time for services. Public center had significantly shorter waiting time than private center. This finding differs from a study that showed a significantly longer waiting time in public health facilities than private ones.[21] The delay waiting time in the private center resulted in dissatisfaction among their clients compared to public center. And it is in agreement with studies where reduction of waiting time to 30 minutes was more important to clients than prolongation of consultation times.[22,23]
An important indicator for continuity of care is whether provider has method to determine client opinion /feedback and gives instruction for follow –up.[16] Only the private center has a method to determine client opinion and feedback through the use of Clients' suggestion box. Providers at both centers performed well with 98.3% (118/120) each for follow up appointment, a study done in Nigeria gave a similar result, where measures to encourage continuity of care were maintained in about 91% of clients.[24]

An important indicator for technical competence is Provider counseling and communication skills, where the information exchanged between clients and providers is important. Private providers were observed to have better client-provider interaction. This is in keeping with findings in a comparative study measuring client satisfaction and quality of FP services in public and private health facilities in Tanzania, Kenya and Ghana.[18]

Indicators of "facility readiness" are used to determine the basic capacity of the facility to provide reproductive health services.[16] Private center having guidelines and supervisory monitoring demonstrate that accepted standards are in place than at the public center. Acceptable procedures and practices are more likely to occur if clinic personnel are able to easily refer to the guidelines. Supervisory visits remind staff of the need to maintain certain

CONCLUSION

The level of satisfaction with FP services offered in both health centers and perceived quality of care based on availability of commodity, observed physical condition of the facilities and providers' behaviors were high. The findings from this study show that providers in private center were rated better than the public counterparts in maintenance of privacy, treatment by other staff, confidentiality and active participation. This is an important aspect of quality of care that increases uptake of family planning methods and continued usage of methods.

Overall, private provider clients were better satisfied with services (93%, 112/120) than clients at public center (88%, 105/120) ($p<0.001$).The private provider was also observed to provide better quality services in some aspects like maintenance of privacy, active participation, treatment by other staff, staff supervision and training.

These facilities need to appropriate standards so as to include all basic elements of family planning service provision. Improved quality will increase uptake of FP methods which will benefit the health and well-being of the women and families thereby achieving MDG5.

Effort to improve the public providers' counseling and communication skills on family planning services is recommended. Establishment of more family planning clinics by private organizations can help reduce work load, thereby reducing waiting time.

Strengths and Weaknesses

This study adds to the body of knowledge on the subject matter especially in developing countries where there is dearth of data. Validated tools were used for data collection and centres which cater to the family planning needs of a large proportion of women of women in the study area were used. The results cannot be generalized to the state.

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Table 1: Social demographic characteristics of respondents

Variable	Public n=120 Freq (%)	Private n=120 Freq (%)	Statistic	Df	P-Value
Age(years)					
20-29	13(10.8)	20(16.6)			
30-39	81(67.5)	47(39.2)			
40-49	26(21.7)	45(37.5)			
50-59	0(0)	8(6.7)			
Mean no± SD	35.5(5.5)	37.9(7.5)	t-stat=0.86	238	p<0.001*
Marital status					
Married	119(99.2)	112(93.3)	x^2=5.66	1	p=0.020*
Single	1(0.8)	8(6.7)			
Educational Status					
Primary	4(3.3)	13(10.8)	x^2=5.62	2	p=0.060
Secondary	64(53.4)	54(45.0)			
Higher	52(43.3)	53(44.2)			
No of children					
0-4	109(90.8)	95(79.2)	x^2=6.41,	238	p=0.010*
>5	11(9.2)	25(20.8)			

*Statistically significant

Table 2: Contraceptive methods received by respondent

Methods received	Public n=79 Freq (%)	Private n=84 Freq (%)
New Clients	**n=34**	**n=26**
Pills	3(8.8)	5(19.2)
IUD	10(29.4)	9(34.6)
Injectable	6(17.6)	7(27.0)
Norplant	14(41.2)	5(19.2)
Condom	1(3)	0(0)
Re-Visit Clients	**n=45**	**n=58**
Pills	7(15.6)	14(24.1)
IUD	4(8.9)	2(3.5)
Injectable	28(62.2)	39(67.2)
Norplant	4(8.9)	3(5.2)
Condom	2(4.4)	0(0)

Table 3: Respondent's rating of the clinic

Variables	Public n=120 Freq (%)	Private n=120 Freq (%)	Statistics	df	p-value
Waiting time (minutes)					
<30					
30-60	68(56.7)	9(7.5)			
61-90	50(41.7)	69(57.5)			
>90	2(1.6)	33(27.5)			
Mean waiting time	0(0)	9(7.5)			
	24.8(11.7)	48.6(17.8)	t-stat=3.29	238	p<0.001*
Perception of waiting time					
Short	10(8.3)	1(0.8)	x^2 =16.82	2	p<0.001*
Moderate	95(79.2)	83(69.2)			
Long	15(12.5)	36(30.0)			
Privacy maintained					
Yes	115(95.8)	117(97.5)	x^2=0.52	1	p=0.470
No	5(4.2)	3(2.5)			
Treatment by other Staff					
Very well			x^2=52.77	2	p<0.001*
Well	4(3.3)	9(7.5)			
Not well	73(60.8)	111(92.5)			
	43(35.8)	0(0)			

*statistically significant

Table 4: Summary of indicators of quality of family planning services as perceived by respondents

Variables	Public n=120 Freq (%)	Private n=120 Freq (%)	X^2	df	p-value
Provider					
Asked client about reproductive intensions	110(91.7)	84(70.0)	18.57	2	P<0.001*
Mentioned STI/AIDS initiates or responds	29(24.2)	51(42.5)	12.46	2	P=0.020*
Discussed Dual method use	20(16.7)	15(12.5)	2.56	3	p=0.460
Gave instruction on how the method accepted works	75(94.9)	81(97.6)	3.46	3	p=0.180
Gave instruction on when to return	118(98.3)	118(98.3)			
	(n=76)	**(n=93)**			
Asked clients if she has any problems(re-visit clients)	72(94.7)	81(87.1)	2.85	1	p=0.050*
Other staff					
Treated client with dignity and respect	77(64.1)	120(100)	52.77	2	P<0.010*
Client					
Participated actively in discussion & selection of method(is empowered)	115(95.8)	120(100)	5.106	2	p=0.020*
Believed the provider will keep her information confidential	116(96.7)	119(99.2)	1.838	1	p=0.180
	(n=80)				
Received her method of choice	56(66.3)	**(n=83)** 60(72.3)	9.91	2	p=0.010*
Facility					
Offers Privacy for clients	115(95.8)	117(97.5)	0.517	1	p=0.470
Has acceptable waiting time	105(87.5)	84(70)	16.82	3	p<0.001*

*Statistically significant

Table 5: Summary of indicators of quality of family planning services as observed during client-provider interaction

Aspect of client provider interaction	Public n=20	Private n=20
Counseling and communication skills	18	19
Information discussed	18	19
performed clinical procedures according to guidelines for Injectable	19	20
performed clinical procedures according to guidelines for pelvic examination	18	19
performed clinical procedures according to guidelines for IUD insertion	**n=10** 9	**n=10** 10

KNOWLEDGE AND ATTITUDES TOWARDS MOBILE PHONE USE TO PROMOTE MATERNAL AND CHILD HEALTH AMONG WOMEN IN MUSHIN, LAGOS STATE

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ABSTRACT
Mobile phone technology has been demonstrated to promote maternal and child health in resource poor settings with slow progress to achieving Millennium Development Goals. However, its benefits are yet to be fully explored in Nigeria. The aim of this study was to determine the knowledge and attitudes towards the use of mobile phones to promote maternal and child health among women of reproductive age group (15 – 49 years) in Mushin, Lagos, Nigeria. In this descriptive cross-sectional survey, data was collected using an interviewer-administered questionnaire from 400 respondents who were selected by multistage sampling method. The mean age of respondents was 28.1± 9.5years and 51% were married. Majority (93.5%) owned a mobile phone but only 23.5% subscribed to short message service (SMS) about health. About half of the respondents had good overall knowledge (51.3%) and positive attitudes (51%). Younger age and higher education were significantly associated with good knowledge and also significantly associated with positive attitudes ($p<0.05$). Although most women owned a mobile phone, knowledge and attitudes towards its use to promote maternal and child health were limited. Therefore, an increased enlightenment of its use is necessary as well as strengthening the partnerships between the health sector, community and the telecommunications industry.

Keywords: *Maternal health, child health, reproductive age, mHealth*

INTRODUCTION

Globally, maternal deaths are unsatisfactorily high. About 287,000 women died worldwide in 2010 due to pregnancy and pregnancy related conditions, 56% of which occurred in developing countries, especially Africa with Nigeria accounting for 14% of global maternal mortality ratio. Most women need access to health facilities, access to antenatal care, skilled health professionals and support, prevention of unwanted or too early pregnancies, access to family planning, safe abortion services to the extent of the law and adequate post abortion care. Daily, Nigeria loses about 2,300 under five year old children and 145 women of childbearing making it the second largest contributor to under-five and maternal mortality worldwide. Majority of the child deaths in the country are usually due to preventable causes. There is thus an urgent need to improve the maternal and child indices in the country using innovative approaches such as mobile health (mHealth), which is the use of mobile communication and network technology for health care use.

mHealth is user-friendly and provides information about health risks, good health behaviors, available resources to mothers and children; enforcing new habits using text messages, pictures and videos to express the desired behavior, health monitoring and surveillance, guiding them to embrace new healthy behaviors through monitoring, feedback, reminders, encouragement and reward giving; promoting intersectoral communication for social support and better access to health facilities.[6] Short message service (SMS) is an inexpensive method of communication and an effective medium to progress mHealth.[7]

With over six billion mobile phone subscribers around the world, mobile phone telecommunication technology has penetrated the world including the low and middle-income

countries and has become an important tool in improving health quality and saving lives. This also creates an opportunity to achieve the fourth and fifth millennium development goals (MDGs) of reducing infant mortality rate and improving maternal health respectively by 2015.[6] Mobile phone use has been demonstrated to improve preparedness for motherhood and enhance healthy behavior among low-income pregnant women in America.[8] It has also improved health knowledge, utilization of antenatal care and immunization services and reduced delays in seeking health care for pregnancy-related complications in less developed countries.[9-11]

In Nigeria, a recent and on-going mHealth intervention in Ondo state, the Abiye project has recorded success in reducing maternal mortality and has improved access to health information, communication between health care providers and pregnant women and improved clients' satisfaction with maternal health services.[12] However, despite the potential benefit of mobile phone technology in promoting maternal and child health particularly in resource limited setting, there is still a paucity of evidence in our setting to adequately inform policy change in this direction. We evaluated the knowledge and attitudes of women of reproductive age group towards the use of mobile phone technology in promoting maternal and child health. The study findings, which project the views of end-users of future mHealth interventions, will serve as a guide for program managers and researchers.

METHODS

Study Area

Mushin, an urban local government area (LGA) where this study was carried out is located in the heart of Lagos State and had a population of 637,341women as at the 2006 census. The LGA has 19 wards and majority of the residents are market traders, factory workers and manual workers. Several of the women are housewives and petty traders. Mobile phone usage is well established in the LGA with good network connectivity to all the major network providers.

Sampling Methodology

A sample size of 400 was calculated using formula $n = z^2pq/d^2$ and assuming maximum variability. Multistage sampling method was used to select respondents. First, one ward in the LGA was selected by simple random sampling. Second, ten streets in the ward were selected by simple random sampling. Third, the habitable houses on each street were enumerated (there was an average of 25 habitable houses on each street) and one household was selected in each house by simple random sampling and one eligible respondent was selected per household. If there was more than one eligible person, simple random sampling was used to select the respondent. A few houses did not have an eligible respondent, in that event, the next house was used.

Data Collection and Analysis

A structured, interviewer-administered questionnaire developed from previous literature[13,14] was used to collect data. Ten trained interviewers who were 300 and 400 level medical students assisted with data collection after the questionnaire was pre-tested in Yaba LGA. The questionnaire collected information on socio-demographic data, mobile phone utilization, knowledge of mobile phone use to promote maternal and child health and attitudes towards mobile phone use to promote maternal and child health.

Collected data was analyzed using Epi-Info software statistical package version 7. Chi-squared test was to determine associations between socio-demographic variables and respondents' knowledge and attitudes. Level of significance was set at 0.05.

To determine composite scores for level of knowledge, each correct response to 8 knowledge questions was given a score of 1 while incorrect/don't know responses were given a score of 0. Respondents with aggregate scores below the mean score were classified as having poor

knowledge and those with aggregate scores above the mean score were classified as having good knowledge. The respondents' attitudes were assessed using a 5 point Likert scale for 10 attitude statements. The overall attitude scores were graded as positive attitude for aggregate of 50% and above and negative attitude for less than 50%.

Ethical Consideration

Ethical approval was obtained from the Health Research and Ethics Committee of Lagos University Teaching Hospital. In addition, permission was obtained from the Medical Officer of Health of the Local Government Area before the study was carried out. Written informed consent was obtained from the respondents and their confidentiality was maintained by not using identifiers.

RESULTS

The modal age group was 15-25years and the mean age was 28.1years ± 9.5 SD. Most of the respondents were married (51%), Christians (62%), Yoruba (80.8%) and had secondary school education (58.3%) [Table 1].

Majority (93.5%) of the respondents owned a mobile phone and 57.8% of the respondents' phones were smart phones. Twenty-eight respondents (7.0%) were subscribed to routine text messages on health, 12(3.0)% had health applications on their mobile phone and 6(1.5%) had ever visited health related websites on phone (Figure 1).

The commonest sources of health-related information for respondents were health care providers (50.8%) and electronic media (44.3%). Most (59.3%) of the respondents knew healthy pregnancy tips could be gotten through mobile phone, 61.3% knew immunization information could be gotten through mobile phone, 78.3% knew antenatal appointment reminders could be gotten through mobile phone, 79.8% knew immunization reminders could be gotten by text message, 68.5% knew reproductive health information could be gotten through mobile phone, 84.3% knew mobile phones could make it easier to reach healthcare providers in case of emergencies, 70.3% knew mobile phone applications and 73.5% knew internet can be used to get information that promotes maternal and child health (Table 2).

Respondents most preferred source of health-related information was the healthcare provider 49.30%. Most (66%) of the respondents felt immunization reminders via mobile phone would be very beneficial. In addition, 76.3% of respondents preferred a reminder via text message to e-mail and 54.8% preferred a reminder via phone call to text message. Most (66.8%) of the respondents strongly agreed that women should seek health information. More (48.5%) respondents strongly agreed that health information should be available to everyone and 42.5% strongly agreed that health information aids better health decisions. More (40.5%) of the respondents strongly agreed that a doctor's advice cannot be replaced by health information obtained from the mobile phone, 15.8% strongly agreed that health information gotten from mobile phones is not reliable, 41.5% agreed that health information through mobile phone was cheap. More of the respondents (30%) agreed that mobile phone usage had increased their level knowledge on various health conditions. More (42.3%) agreed that mobile phone is a good way to access health information and in addition, 36.3% strongly agreed that they would use their phones to get health information. More of the respondents (38.5%) strongly agreed to encourage others to get maternal and child health information through their phones (Table 3).

The mean knowledge score of respondents was 5.8 ± 2.5 SD. Overall, 51.3% of the respondents had good knowledge while 48.8% had poor knowledge on mobile phone use to promote maternal and child health. There was statistically significant association between education and

level of knowledge as higher proportions of respondents with secondary and tertiary education had good knowledge ($p<0.001$). There was also a statistically association between age and level of knowledge as higher proportions of respondents in the younger age groups had good knowledge ($p = 0.025$) [Table 4].

Overall, 51% of respondents had a positive attitude and 49% had a negative attitude towards mobile phone use to promote maternal and child health. Higher proportions of respondents with secondary and tertiary education had positive attitude; higher proportions of respondents in younger age groups had positive attitude and a higher proportion (62.9%) of respondents with good knowledge had positive attitudes. These findings were statistically significant ($p < 0.05$) [Table 4].

DISCUSSION

There are currently over 170 million subscribers to the Global System Mobile communication (GSM) service in Nigeria.[15] The use of mobile phones to promote maternal and child health has great potential as the number of subscribers keeps increasing. The ubiquitous existence of the mobile phones explains why almost all (93.5%) the respondents in the study owned one. Similarly, a facility-based study among women accessing care at an immuno-prophylaxis and child welfare clinic in Lagos University Teaching Hospital (LUTH), a tertiary hospital within Mushin LGA revealed that 98% of the women had mobile phones.[13]

The smart phone has the ability to connect to the Internet on the go. In this study, 57.8% of respondents owned a smart phone; this could be because of higher costs and complexity compared to regular phones or access to Internet from other sources although that information was not sought in this study. Very small numbers of respondents were subscribed to routine health SMS, had health applications on phone or had visited health websites on their phones thus showing a poor use of mobile phone to access health information. With the respondents' mean age of 28 years, one would have expected a higher use of mobile phone to access health-related information as younger age has been linked with higher likelihood of seeking health related information over the Internet.[16] A population study in Japan also demonstrated low use (6%) of mobile phone to access health-related information.[17] However, 22% of migrants studied in Australia accessed health information via mobile phone. [14] Also, among undergraduates in USA where 52.4% used smart phones, a higher proportion than in our study (17%) had used an mhealth application on the phone within the last 30 days.[11] It is worthy of note that such applications are not as readily available in Nigeria as in developed countries such as USA.
The respondents' most common source and the most preferred source of health information were health care providers. Factual health information can be obtained from this source. However, in practice, the information obtained from health care providers may not meet the patients' needs especially when care is not patient-centered resulting in patients seeking additional health information through telecommunications.[18]

Only about half of respondents had good knowledge of the use of mobile phone to promote maternal and child health. This could be because of their unfamiliarity with using mobile phones to access health information. A study among kidney transplant patients in USA also demonstrated poor knowledge of the mobile phone use for remote monitoring (7%) although 90% of the respondents owned a mobile phone.[19] A possible implication of not having adequate knowledge is that these end-users of future mHealth interventions to promote maternal and child health may fail to appreciate the benefit of such interventions and may not participate fully in them. This point is underscored in this study as good knowledge was significantly associated with positive attitude towards mobile phone use to promote maternal and child health.

Most (66%) respondents appreciated the benefit of immunization appointment reminders via mobile phone but 54.8% would prefer phone calls to SMS. Similarly, 69% of the mothers in the LUTH study perceived the reminders to be very beneficial and 67% preferred phone call reminders.[13] This could be because SMS is more impersonal than a phone call; future interventions should put into consideration the preference of end-users of mHealth services.

Similar to overall knowledge, only about half of the respondents had positive attitude. Some previous studies in USA had higher proportions of respondents that were receptive to mHealth interventions.[19, 20] The preference for health information from health care providers could have contributed the attitudes observed in our study. For instance, over 80% felt health information should not be available to everyone, about 40% believed health information via mobile phone is not reliable and about 70% felt heath information over phone cannot replace a doctor's advice. Although, provider-patient communication in the developed world is being complemented significantly with online health information[21], there seems to be more confidence in health care providers and less trust in online information in our study. Future mHealth interventions should thus seek to get the endorsement of health care providers in order to ensure their usage within communities.

Younger age and higher education were significantly associated with both good knowledge and positive attitude. Younger and better-educated people are the ones who tend to use mHealth technology.[16] Thus, they are likely to know more about its use and have positive attitudes towards it. In relation to this study, it underscores the importance of female education, which has been shown to advance maternal and child health.[22]

CONCLUSION

Although, almost all the respondents in this study owned a mobile phone, knowledge and attitudes towards its use to promote maternal and child health were limited. The health information-seeking behaviour was very low and respondents relied majorly on healthcare providers for health related information. Therefore, an increased enlightenment of its use is necessary as well as strengthening the partnerships between the health sector, community and the telecommunications industry.

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Table 1: Socio-demographic characteristics of respondents

Variables	Frequency (%) n=400
Age group (years)	
15-25	190 (47.5)
26-35	125 (31.3)
36-45	58 (14.5)
>45	27 (6.8)
Marital status	
Single	182 (45.5)
Married	204 (51.0)
Divorced	8 (2.0)
Widowed	6 (1.5)
Religion	
Christianity	248 (62.0)
Islam	151 (37.8)
Traditional	1 (0.3)
Tribe	
Hausa	4 (1.0)
Igbo	45 (11.3)
Yoruba	323 (80.8)
Others	28 (7.0)
Educational Level	
None	5 (1.3)
Primary school	67 (16.8)
Secondary school	233 (58.3)
Tertiary education	94 (23.5)
Quaranic school	1 (0.3)

Table 2: Sources of health-related information and knowledge of mobile use to promote maternal and child health

Variables	Frequency (%) n=400
***Sources of health-related information**	
Newspaper/magazine	59 (14.8)
Television/radio	177 (44.3)
Research article/ Journal	17 (4.3)
Friends/Family	101 (25.3)
Mobile phones	107 (26.8)
Healthcare providers	203 (50.8)
Healthy pregnancy tips could be gotten through mobile phones	
Yes	237 (59.3)
No	87 (21.8)
Don't know	76 (19.0)
Immunization information could be gotten through mobile phone	
Yes	245 (61.3)
No	91 (22.8)
Don't know	64 (16.0)
Antenatal appointment reminder could be gotten through text message	
Yes	313 (78.3)
No	61 (15.3)
Don't know	26 (6.5)
Immunization reminders could be gotten through text message	
Yes	319 (79.8)
No	44 (11.0)
Don't know	37 (9.3)
Reproductive health information could be gotten through mobile phone	
Yes	274 (68.5)
No	53 (13.3)
Don't know	73 (18.3)
Mobile phones could be used to reach healthcare providers in case of emergency	
Yes	337 (84.3)
No	36 (9.0)
Don't know	27 (6.8)
Phone applications could be used to get information that promotes maternal and child health	
Yes	281 (70.3)
No	54 (13.5)
Don't know	65 (16.3)
The Internet could be used to get information that promotes maternal and child health	
Yes	295 (73.8)
No	40 (10.0)
Don't know	65 (16.3)

*Multiple responses allowed

Table 3: Respondents' attitudes towards health information via mobile phone

Variables	Frequency (%) n=400
Perceived benefit of immunization reminder via mobile phone	
Very beneficial	264 (66.0)
Somewhat beneficial	100 (25.0)
Prefers reminder via text message to e-mail	305 (76.3)
Prefers reminder via phone call to text message	219 (54.8)
Women should seek health information	
Strongly agree	267 (66.8)
Agree	118 (29.5)
Not sure	7 (1.8)
Disagree	8 (2.0)
Health information should be available to everyone	
Strongly agree	194 (48.5)
Agree	164 (41.0)
Not sure	23 (5.8)
Disagree	18 (4.5)
Strongly disagree	1 (0.3)
Health information helps to make better health decisions	
Strongly agree	170 (42.5)
Agree	196 (49.0)
Not sure	25 (6.3)
Disagree	9 (2.3)
Health information got through mobile phone cannot replace a doctor's advice	
Strongly agree	162 (40.5)
Agree	123 (30.8)
Not sure	50 (12.5)
Disagree	57 (14.3)
Strongly disagree	8 (2.0)
Health information through mobile phone is not reliable	
Strongly agree	63 (15.8)
Agree	97 (24.3)
Not sure	149 (37.3)
Disagree	79 (19.8)
Strongly disagree	12 (3.0)
Health information through mobile phone is cheap	
Strongly agree	48 (12.0)
Agree	166 (41.5)
Not sure	138 (34.5)
Disagree	42 (10.5)
Strongly disagree	6 (1.5)

Table 4: Factors associated with overall knowledge and attitude of respondents

Variables	**Overall knowledge**		**X^2**	**p - value**
	Good Frequency (%)	**Poor Frequency (%)**		
Age group (years)			9.31	**0.026**
15-25	104(54.7)	86(45.3)		
26-35	68(54.4)	57(45.6)		
36-45	26(44.8)	32(55.2)		
>45	7(25.9)	20(74.1)		
Total	205 (51.3)	195 (48.8)		
Level of education				**<0.001***
Primary school	19(28.4)	48(71.6)		
Secondary school	117(50.2)	116(49.8)		
Tertiary education	68(72.3)	26(27.7)		
Quaranic school	0(0)	1(100)		
None	1(20)	4(80)		
Total	205 (51.3)	195 (48.8)		
	Overall attitude			
	Positive Frequency (%)	**Negative Frequency (%)**		
Age group (years)			12.53	**0.005**
15-25	108(56.8)	82(43.2)		
26-35	66(52.8)	59(47.2)		
36-45	23(39.7)	35(60.3)		
>45	7(25.9)	20(74.1)		
Total	204 (51.0)	196 (49.0)		
Level of education				**<0.001***
Primary school	18(26.9)	49(73.1)		
Secondary school	129(55.4)	104(44.6)		
Tertiary education	56(59.6)	38(40.4)		
Quaranic school	0(0)	1(100)		
None	1(20)	4(80)		
Total	204 (51.0)	196 (49.0)		
Overall knowledge			23.94	**<0.001**
Good	129 (62.9)	76 (37.1)		
Poor	75 (38.5)	120 (61.5)		
Total	204 (51.0)	196 (49.0)		

*****Fishers' exact p-value

KNOWLEDGE OF HEALTH EFFECTS OF EARPHONE USE AMONG MEDICAL STUDENTS OF COLLEGE OF MEDICINE, UNIVERSITY OF LAGOS

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ABSTRACT

Background: One of the major consequences of exposure to loud sounds is a phenomenon called **noise induced hearing loss** (NIHL). Using headphones at high volumes can cause temporary or permanent hearing impairment or deafness. There is yet paucity of information available on studies conducted on earphone usage and its effect on hearing in Nigeria. This study therefore aimed at assessing the awareness of medical students of College of Medicine University of Lagos (CMUL) on the health effects of earphone usage. **Methods**: This cross-sectional descriptive study employed a multi-stage sampling method in respondents' selection. Data was collected using a structured, self-administered questionnaire, and analyzed using EpiInfo version 3.5.1. **Results**: The majority of respondents (90%) had heard of noise-induced hearing loss (NIHL) before. Slightly more than half (54.2%) knew that both excessive listening volumes and listening for prolonged periods could predispose to hearing loss. Almost nine of every ten respondents (86.3%) had ever used earphones to listen to music, with about 40% of listeners reporting listening volumes as loud or very loud. A quarter of respondents (25.1%) had experienced ringing sensation in the ears during the course of earphone use. **Discussion**: NIHL is a condition that the majority of respondents had heard of although some did not have correct knowledge about it. The practice of listening using earphones for prolonged periods or at loud volumes was quite common among respondents, which is dangerous to hearing. The study recommends the promotion of health education about the adverse effects of inappropriate usage of earphones.

***Keywords**: Earphone usage, social noise, hearing loss, Noise-induced hearing loss.*

INTRODUCTION

Hearing loss is any change in hearing acuity in quiet or in the presence of background noise, but can be quantified in an audiogram as an auditory threshold of greater than 15dB at any frequency.[1] One of the major consequences of exposure to loud sounds is a phenomenon called noise induced hearing loss (NIHL). Noise-induced hearing loss is a form of hearing loss caused by sustained and repeated exposure to excessive sound levels. While commonly attributed to prolonged employment in high-noise industries, any form of sound exposure can lead to NIHL provided there is sufficient intensity and exposure time.[2] This type of hearing loss is 'sensori-neural', occurring because of damage to the hearing organ (cochlea) of the inner ear.[3,4]

NIHL can be caused by a one-time exposure to an intense "impulse" sound, such as an explosion, or by continuous exposure to loud sounds over an extended period of time, such as noise generated in a woodworking shop.[5] Exposure to sound above a level of approximately 85dB initially manifests as a temporary hearing loss or 'dullness' of hearing (*a temporary threshold shift*) which recovers within 16–24 hours of the exposure. However, with repeated or sustained exposure, the hair cells and associated nerve fibres degenerate and the threshold shift becomes permanent (*permanent threshold shift*).[2] Long or repeated exposure to sounds at or

above 85 decibels can cause hearing loss.[6] Potential for hearing loss depends on volume settings, duration of listening session, intensity, and frequency of exposure. NIHL from both impulse and continuous noise can be prevented by regularly using hearing protectors such as earplugs or earmuffs.[7,8] Noise continues to be the largest compensable occupational hazard. Occupational noise exposure is a decreasing hazard, but still substantial, whereas social noise is increasing as a hazard for young people as occupational noise decreases.[9,10] Major sources of social and leisure noise include rock concerts, personal stereos and other portable media using earphones.

Using headphones at a sufficiently high volume level can cause temporary or permanent hearing impairment or deafness due to an effect called "masking." The headphone volume has to compete with the background noise, especially in excessively loud places such as subway stations, airports, and large crowds. This leads to the disappearance of the normal pain associated with higher levels of volumes. Extended periods of the excessively loud volume may be damaging.[10,11] It is reported that approximately 22 million Americans between 20-69 years of age have experienced permanent damage to their hearing from overexposure to loud noises at work or during leisure activities.[12] People of all ages, including children, teens, young adults, and older people, can develop NIHL.[13]

Portable music players have continued to increase in popularity especially among young people, and these listening devices have become smaller and more sophisticated. Battery span has also been elongated, meaning that users can listen to music and other sounds for longer durations. Earphones have been packaged to deliver sound at high volumes into the ear. A 2006 national study of 1000 individuals aged 18 to 70 years conducted for the American Speech-Language-Hearing Association provides information on the contemporary listening habits of youth and adults. This study found that approximately 36 percent of adults and 62 percent of students used Walkman personal electronic listening devices, 11 and 36 percent respectively used iPod devices, and 11 and 25 percent respectively used other brands of MP3 players.[14] One reason cited for their increased popularity is that users can 'escape' from their physical environment while at the same time personalizing their listening environment; resulting in increasing social isolation and personalization.[15]

There is paucity of information available on studies done on either earphone usage or its effect on hearing in Nigeria. Most studies on hearing loss were centered on occupational causes or childhood hearing loss. The danger here is that the use of earphones as a contributor to noise pollution and particularly, hearing loss or impairment has been largely neglected. There is limited awareness on the dangers of prolonged usage at high volumes, and there are no policies regulating the maximum sound intensities an earphone should be capable of producing. Worst of all, the temporary hearing loss which is the major consequence of this habit may occur insidiously and eventually become permanent and irreparable.[15]

This project therefore aimed at determining the usage of portable media devices and earphones as well as assessing the level of awareness of medical students at the College of Medicine, University of Lagos (CMUL) on the long term consequences of prolonged usage of earphones especially at high volumes. It is hoped that the results will provide data on the knowledge of NIHL, and the need to take steps to enlighten the populace about this subtle threat to hearing.

METHODS

Description of Study Area

The College of Medicine, University of Lagos was established in April, 1962 and is located within Mushin Local Government Area of Lagos state, sharing the premises with the Lagos

University Teaching Hospital (LUTH). The College buildings are within. The College awards degrees in Medicine & Surgery (MBBS), Dentistry (BDS), Physiology, Microbiology, Physiotherapy, Radiography (B.Sc.). The population of medical students as at 2009 was 600. Students have the option of residing within the hostels or attending lectures as day students.

Study Type
It was a descriptive cross-sectional study designed to determine the awareness of medical students in the College on the health effects of inappropriate usage of earphones on hearing.

Sample Size Estimation
The sample size was calculated using the formula: $\mathbf{n= Z^2pq/d^2}$ and then corrected taking into account that the total population of students was less than 10,000. A minimum sample size of 234 was obtained, and this was increased by 10% and rounded up to 260 participants to allow for non-response.

Sampling Method
Multi-stage sampling method was employed in the selection of study subjects. Firstly, the study population was divided into strata based on the current year/class of study of each student. Step 2 involved a proportionate sampling of respondents in each of the strata. Systematic sampling was then used to select members of each class who would be included in the study.

Data Collection
A Structured self- administered questionnaire was used to collect data on demographics, knowledge, attitude and practices of respondents in relation to noise-induced hearing loss.

Data Analysis
Data were analyzed by computer using EPI Info version 3.5.1. and data was presented as frequency tables.

Ethical Considerations
All participants in the study were duly informed of the nature and purpose of the study, after which verbal consent was obtained from willing participants. Respondents were assured of strict confidentiality and were not required to provide personal identifiers.

RESULTS
Of the 260 questionnaires administered, 240 were satisfactorily filled and returned, giving a response rate of 92.3%.

The majority of respondents were aged between 16 and 24 years (88.8%), not married (97.5%), Christian (87.5%) and of Yoruba ethnic group (75%). There were slightly more male respondents (55%) than female (45%) (Table 1)
The majority of respondents (90%) had heard of noise-induced hearing loss before (Table 2).

Only about one-quarter (25.9%) of respondents who had heard of NIHL knew that it could be caused by a single exposure to loud sounds. Slightly more than half (54.2%) of respondents knew that both excessive volumes of listening and listening for prolonged periods are conditions necessary for noise-induced hearing loss. The majority of respondents (184, 85.2%) knew that earphone usage could predispose to NIHL (Table 3).

The majority of respondents (69.4%) knew that difficulty in hearing normal conversation was a symptom of hearing loss, but only a third (33.8%) knew that difficulty hearing in noisy environment was also a symptom (Table 4).

Table 5 shows that almost 90% of all respondents had used earphones to listen to music. The most popular type of earphone used was earplugs (70.5%). About 30% of earphone users listened for more than one hour at a stretch, and about 40% reported listening volumes as loud or very loud. Three-quarters of earphone users expressed some concern of possible hearing loss as a result of its use.

Among earphone users, the predominant condition experienced was difficulty in hearing while using earphones (59.4%) (Table 6).

DISCUSSION

Noise Induced Hearing Loss from exposure to leisure activities such as music is a phenomenon that is largely unstudied in this part of the world as evidenced by the paucity of studies on the topic. The study was carried out to determine the level of awareness of medical students of the College of Medicine, University of Lagos on the effects of inappropriate usage of earphones on hearing.

The study revealed that NIHL is a phenomenon that the majority of respondents (90%) had heard of although some of them did not have correct knowledge about it. Of the 216 respondents who had heard of NIHL, the majority (87%) knew that NIHL could result from repeated exposure to loud sounds. However, only a quarter (25.9%) knew that NIHL could be caused by a single exposure to loud sounds- this reflects either an incomplete knowledge of NIHL or an erroneous knowledge that a single exposure to excessive sound levels could not cause hearing loss. This however does not compare with findings from a similar study among South African University students, in which less than forty percent (38%) had heard of NIHL[16]. Knowledge of symptoms of hearing loss among the medical students varied from 69.4% for difficulty in hearing normal conversation, to 33.8% for difficulty hearing in noisy places. This shows that a large proportion of respondents did not know some of the signs and symptoms of hearing loss, and thus may not be able to take necessary steps to arrest the progression of hearing loss which they may experience over time from exposure to noise.

A large percentage of respondents (85%) knew that earphone usage could predispose to hearing loss although about one in five respondents who used earphones (25.8%) were not concerned about experiencing hearing loss from earphone usage. This finding differs from that in the study among South African university students, in which only 29% of respondents were aware of the possibility of hearing loss due to the use of personal audio devices, and two-thirds (65%) showed little or no concern about how the use of these devices might affect their hearing.[16] However it should be noted that our study was conducted among medical students, and this could explain the wide disparity in their knowledge.

Regardless of the type of listening device, most respondents in this study (68.1%) listened using earphones for 30-60 minutes at a stretch, whereas the typical listening session in another study among teens and adults lasted from 1 to 4 hours for approximately 40% of adults and 30% of students.[17] Results from the South Africa study were however comparable to ours, in which 58%, of respondents listened to their devices for an hour or less per session (as appropriate).[16] The increased length of listening time coupled with high sound levels, which recent earphones are capable of delivering, create conditions that may predispose a listener to hearing loss.[18]

Majority of respondents (44.9%) reported that they listened at moderate loudness while nearly 40% listened at either loud or very loud volumes. A study conducted by *Zogby International* found out that 52% of adults and 46% of students were most likely to set the volume at medium when using earphones for their cell phones. It was also found out that students were only slightly more likely than adults to have the volume set on loud (37% to 30%) or low (18% to 14%).[17] Sixty-two per cent of the respondents from the South Africa study listened to their devices at very soft to medium loud levels, while 38% listened to their devices at somewhat loud to very loud levels.[16] From our study, major reasons for choosing to listen at loud or very loud volumes included 'to drown background noise' (30.9%) and 'to hear the lyrics or rhythm properly' (24.2%). A qualitative study among adolescents in the Netherlands revealed that though they appeared to be generally aware of the risks of listening to music at loud volumes, they expressed low personal vulnerability to music-induced hearing loss. Most of these respondents indicated that they would not be willing to change their music-exposure habits.[19] Guidelines for appropriate listening levels and duration using earphones, and appropriate earphone type are necessary to reduce the risk for NIHL.[15]

Over half of respondents (59.4%) had difficulty in hearing while listening with earphones, and needed to be shouted at in order to get their attention. This shows that the respondents may either have been listening at loud or very loud volumes, or may have been using canal earphones which have the capability to reduce ambient sound levels entering the ear. Interestingly, one quarter of respondents in this study (25.1%) had experienced ringing sensation in the ears during the course of using earphones. In the study conducted in South Africa among university students, 44% of the participants reported some kind of hearing-related problems, most common of which was ringing in the ears (33%). Only one person reported actual hearing loss in that study.[16]

CONCLUSION

The study revealed a high level of awareness of NIHL among the medical students, although a smaller proportion of them knew that NIHL could be caused by either a single or repeated exposures to loud sounds. There was a high level of awareness on the auditory effects of inappropriate usage of earphones as many knew that it could predispose to hearing loss.

The majority of medical students demonstrated a fairly appropriate usage of earphones in listening to music, although a small proportion listened for durations and volumes that may predispose to hearing loss. The practice of listening using earphones in noisy settings was quite common and majority of such listeners had to turn up the volumes of their devices to hear well. This practice is dangerous to hearing as the increase in volumes beyond safe levels predispose to hearing loss as mentioned in the discussion. Although three-quarters of respondents were either concerned or very concerned about the possibility of hearing loss from earphone use, it is not known if any actions were taken by respondents to reduce the risks posed by earphone use to their hearing.

From the results, it is clear that there is need for promotion of health education and awareness on the potential dangers of inappropriate earphone usage.

RECOMMENDATIONS

In the light of the findings from this study, the following are recommended:

1. Promotion of health education to inform earphone users about the adverse effects of inappropriate usage of earphones. Users should be alerted as to the dangers that listening at loud volumes and for prolonged periods may pose to their hearing. This can be done through

the mass media and by introducing the topic into school curriculum and lectures at the early stages of education.

2. Promotion of awareness of the availability of special technology to reduce the sound levels delivered into the ear. Special earphones exist to help minimise the potential damage caused by sound entering the ear directly and they include: Noise cancelling headphones and Canal earphones which enable listeners to listen at lower volumes safe for the ear even in noisy settings.
3. Advocacy for legislation. The government should either compel manufacturers of music devices to set a limit on the maximum volume their devices can produce or ensure that they include a package (software or health education leaflets) that enlightens the device user on the adverse effects of listening at loud volumes and for prolonged periods. The device software could inform the user of the maximum listening time allowed for a particular volume level chosen and warn the user when safe levels are being exceeded.
4. Advocacy for preventive health. There should be increased awareness of the need to do routine screening tests so as to be able to detect hearing loss at its insipient stages and thus take measures to prevent further damage.

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Table 1: Socio-demographic Characteristics Of Respondents

Socio-demographic characteristic	**Frequency (n=240)**	**%**
Age (years)		
16-18	38	15.8
19-21	106	44.2
22-24	69	28.8
25-27	15	6.2
>27	12	5.0
Gender		
Male	132	55.0
Female	108	45.0
Marital status		
Married	6	2.5
Not married	234	97.5
Religion		
Christianity	210	87.5
Islam	30	12.5
Ethnicity		
Yoruba	176	73.3
Igbo	46	19.2
Hausa	4	1.7

Table 2: Respondents Who Have Ever Heard Of *'Noise-Induced Hearing Loss'*

Ever heard of NIHL	**Frequency**	**%**
Yes	216	90.0
No	24	10.0
Total	240	100.0

Table 3: Knowledge Of Causes And Necessary Conditions For Nihl

Causes of NIHL (n=216)*	**FREQ**	**%**
A single exposure to loud sounds	56	25.9
Repeated exposure to loud sounds	188	87.0
Conditions necessary for NIHL	**FREQ**	**%**
Excessively loud volumes	86	39.8
Listening for prolonged periods	13	6.0
Combination of excessively loud volumes and prolonged listening	117	54.2
TOTAL	216	100.0

(*Multiple responses allowed)

Table 4: Respondents' Knowledge Of Symptoms Of Hearing Loss

Knowledge of symptoms of hearing loss*	**Frequency (n=216)**	**%**
Buzzing sensation in the ear	121	56.0
Ear discomfort	120	55.6
Difficult hearing in noisy place	73	33.8
Difficulty in hearing normal conversation	150	69.4

(*multiple responses allowed)

Table 5: Earphone Usage By Respondents

VARIABLE	**FREQUENCY**	**%**
Listen to audio with earphones (n=240)		
Yes	207	86.3
No	27	11..3
No response	6	2.4
Type of earphones used *(multiple responses allowed; n=207)		
Canal phones	35	16.9
Earplugs	146	70.5
Headphones	40	19.3
Average listening time at a stretch		
Less than one hour	141	68.1
One to two hours	40	19.3
Two to three hours	25	12.1
More than 3 hours	1	0.5
Perceived volume at which audio is usually listened to		
Very low	11	5.3
Low	22	10.6
Medium/moderate	93	44.9
Loud	60	29.0
Very loud	21	10.1
Reasons for listening at moderate/loud volumes (n=174)		
Drowns background noise	64	36.7
Hear lyrics of music	50	28.7
Helps to concentrate on reading	18	10.3
Sleep well	6	3.4
Just like it	36	20.7
Concerned about hearing loss as a result of earphone use		
Yes	154	74.2
No	53	25.8
Total	207	100.0

Table 6: Hearing Loss Symptoms Experienced By Earphone Users

Symptoms experienced (MRA, n=207)	**Frequency**	**%**
Muffled hearing	42	20.3
Ringing in ears	52	25.1
Pain in ears	41	19.8
Difficulty in hearing	123	59.4
No symptom experienced	56	27.0

KNOWLEDGE AND MEASURES USED IN PREVENTION AND TREATMENT OF HIV/AIDS AND SEXUALLY TRANSMITTED INFECTIONS AMONG NATIONAL UNION OF ROAD TRANSPORT WORKERS MUSHIN, LAGOS STATE

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ABSTRACT
Background: Globally, about three hundred and sixty million new cases of curable STIs occur yearly. HIV/AIDS and other STIs and their complications reduce the quality of life of infected individuals. They also contribute to the overall poor health statistics of developing countries. This study assessed HIV/AIDS and STI knowledge, preventive and treatment measures adopted by National Union of Road Transport Workers (commercial bus drivers division) in Mushin local government, Lagos state. **Methodology:** A cross sectional descriptive study using interviewer-administered semi-structured questionnaires was used. One of the three NURTW divisions in Mushin LGA was randomly selected; all the garages in the selected unit were used. Sample size of 225 was calculated and all the registered drivers available during the period of data collection were interviewed. Data was analyzed using Epi info version 7.1. Association between variables was demonstrated using chi square at $p<0.05$. **Results:** The mean age was 34.89+10.88. Almost all had heard of HIV/AIDS and STI mainly through the media. The commonest STI known were Gonorrhea 98.7%, Syphilis 72.29% and Genital warts 42.42%. For symptoms of STIs, 72.73% knew about genital itching, 88.45% genital discharge, 69.26% genital sores, 90.91% pain during urination. Regarding transmission of HIV and other STI, majority knew that HIV and STIs were preventable, 7.87% knew about abstinence, 5.24% faithfulness to a partner and 77.90% consistent condom use. Most of the respondents knew that STIs could be cured with antibiotics (65.00%). Less than half had good knowledge regarding transmission and prevention of HIV/AIDS and STI. The higher the level of education of the driver the more knowledge they possess ($p=0.036$). About 63.14% of the respondents used condom among which only 9.40% used it consistently. Only 36.86% of the respondents had been screened for HIV, among this group 60.92% got tested in a government facility and only 43.68% had the test with their main sexual partner. About 25.48% of the respondents had treated an STI in the past. Pain during urination was the most common symptom (52.49%), among this group, 22.39% went to a health facility as their first action, more of the respondents (40.30%) were treated by traditional healers and 37.31% chemist. Respondents who used inappropriate STI treatment measure were more likely to have used condom at last sex ($p=0.040$). Single respondents and those who smoked hemp were more likely to use condom, while respondents who did not smoke cigarette or drink alcohol were more likely to be consistent condom users. Respondents between 30-40years of age, who closed late from work, who did not smoke hemp, were married and had good knowledge of HIV/AIDS and STIs were more likely to have been tested for HIV. **Conclusion:** The drivers exhibited poor knowledge with regards to HIV/AIDS and STI, inadequate preventive measures and inappropriate treatment practices. Education and STI counseling as well as HCT services should be available within garages. Peer educators should be trained among NURTW members.

Keywords: *HIV/AIDS, STIs, Knowledge, Prevention, NURTW*

INTRODUCTION

Globally an estimated one million people get infected daily with one of the organisms causing sexually transmitted infections (STI).[1] Most of the infections are curable and are caused by one

of the following organisms: Neisseria Gonorrhea, Treponema Pallidum, Chlamydia Trachomatis and Trichomona Vaginalis. A high proportion is also caused by Human Immunodeficiency Virus, Herpes Simplex Virus and Human Papilloma Virus which are organisms responsible for incurable infection.[1]

In 2008, global incidence of the most common STI increased by more than ten percent compared to 2005. More than half of the reported cases of new STI occurred in males. Over nine million Africans were estimated to be infected with an STI at any particular point in 2008.[1,2]

According to the World Health Organization (WHO) in 2011, an estimated 0.8% of adults aged 15 to 49 years were infected globally with Human Immunodeficiency Virus(HIV) and about 1.7 million deaths were attributed to Acquired Immunodeficiency Syndrome (AIDS) related causes.[2] The disease burden is high in the African region. This region had a high prevalence and mortality rate for HIV among the same age group. The prevalence of HIV among adults aged 15 to 49 years was 4.6% and an estimated 1.2 million people died as a result of being infected with the virus during the same time period.[3]

The first reported case of AIDS in Nigeria occurred in 1986, since then periodic national sero prevalence survey carried out in the country showed a gradually increasing pattern till 2010.[4] WHO estimates that the number of deaths attributable to HIV/AIDS in Nigeria increased from an estimated one hundred and fifty thousand in 2001 to two hundred and ten thousand in 2011.[2]

STI are some of the main diseases for which Nigerians seek medical assessment even though it is estimated that they are underreported and could at times be asymptomatic. HIV/AIDS is one of the ten most notified diseases in Nigeria. High prevalence rate has been observed among sexually active young people this is compounded by inadequate access to diagnostic and treatment services.[5]

Various risk factors for STI transmission have been identified which include unprotected anal or vaginal sex, exchange of contaminated needles and syringes for injecting drugs, presence of a co-existing STI, unsafe blood transfusion, unsterile cuttings or piercings and accidental needle stick injuries.[6]

Studies carried out among commercial drivers in Nigeria and other developing countries showed that commercial drivers exhibit high risk sexual behavior, high prevalence of STI and poor treatment practices.[7-9] In a 2012 study carried out among the general population in Nigeria an estimated seven percent reported symptoms of STI ranging from burning sensation, genital discharge, genital ulcers and genital swelling in the twelve months preceding the study.[10]

The 2013 Nigeria Demographic and Health Survey (NDHS) showed that knowledge of HIV prevention among men in the general population has remained unchanged: in 2008 an estimated sixty eight percent knew that limiting number of sexual partners and condom use prevents STI including HIV, the recent survey recorded seventy percent.[11]
This study was carried out to assess the knowledge and measures used in prevention and treatment of HIV/AIDS and Sexually Transmitted Infections among National Union of Road Transport Workers in Mushin, Lagos State. The result of this study will add to the body of knowledge available for this high risk group of men.

METHODS

Lagos state is the former capital of Nigeria with an estimated population of 17.5 million according to the 2006 state census. Mushin local government area (LGA) is one of the twenty local government areas in Lagos state.[12] Mushin is bounded in the North-west by Oshodi-Isolo LGA, in the East by Shomolu LGA, in the South by Surulere LGA and in the North by Ikeja LGA.[13] The local government is made up of three main divisions; Mushin, Ilupeju and Itire.

The National Union of Road Transport Workers (NURTW) is an entity of the Nigerian Labor Congress (NLC) registered as a trade union in1978. It has an estimated national membership of over 1.5 million. It comprises of all professional commercial drivers, including bus town service or interstate drivers, taxi drivers involved in transportation of passengers and goods from government owned motor parks. The aim of the association is to promote the economic wellbeing of her member's.[14] NURTW in Mushin LGA has commercial bus drivers division both interstate and intra-city, tricycle operators division, motorcyclist division, trailer lorry drivers division and taxi drivers division. The commercial bus driver division 3 branches namely: Odeolowo, Mushin-Ajina and Itire.

This study was carried out using a cross-sectional descriptive design. The Cochran's formula $n=z^2pq/d^2$ was used to calculate sample size of 225 using prevalence of 82.2% (the proportion of market men and women who knew of Abstinence, Being faithful and Consistent condom use as preventive measures against HIV/AIDS transmission in a previous study carried out in Nigeria)[15] Random sampling method was used to select one of the three divisions (Mushin-Ajina division). There are 13 parks/units in Mushin-Ajina namely, Mushin main garage 1 and 2, Palm avenue, Idi oparun 1 and 2, Onilegogoro, Idi-oro, Mushin-Obalende, Dakobiri, Kajola, Mushin total 1 and 2 and Pako junction with 236 registered members. All the registered drivers who were available during the period of the study were interviewed between April and May in 2014.

Information was obtained from the respondents using an interviewer-administered semi-structured pre tested questionnaire. The questionnaire was categorized into three sections; section A elicited the socio-demographic characteristics of the respondents; section B the knowledge of respondents about HIV/AIDS and STIs and section C prevention and treatment modalities for HIV/AIDS and STI. Ethical approval for the study was obtained from the Research and Ethics Committee of Lagos University Teaching Hospital. Permission was obtained from the chairman of National Union of Road Transport Workers of each garage (unit). Written informed consent was also obtained from each respondent. Respondents were assured of confidentiality of information provided and participation was voluntary.

Data entry and analysis was done using EPI Info version 7.0. The data was presented as tables. Mean and standard deviation was computed for continuous variables while frequency was generated for categorical variables. Twenty three knowledge questions were scored and graded; respondents who scored below the mean were graded as having poor knowledge while those with scores above the mean were graded as having good knowledge. Treatment was categorized into appropriate and inappropriate. Going to the hospital as the first line of action being appropriate treatment practice. Association between variables was determined using Chi square at $p<0.05$.

RESULTS

The mean age was 34.89+10.88. More of the respondents (34.75%) were between the ages of 26-35years. Almost two thirds had secondary school education (63.14%) and were married (65.25%). Majority of the respondents resumed in the garage between 4am to 6am (53.39%) and

leave between 8pm to 11pm (57.63%). One-fifth (21.37%) of respondents smoked cigarette among this group majority smoked less than 5 sticks per day. About a quarter (24.68%) smoke hemp with majority smoking it on a regular basis. More than half (59.72%) of respondents consume alcohol and most of them drink alcohol on a regular basis.

Awareness of HIV/AIDS was almost universal, the commonest source of information being television and radio. Almost all (97.87%) the respondents knew that HIV and STIs could be prevented. Regarding modes of transmission, 99.13% knew about unprotected sex, 93.48% knew about blood transfusion, 96.52% knew it can be transmitted through sharing of sharp objects, 57.83% knew that an infected mother can infect her unborn baby while 59.57% knew that breastfeeding can transmit the virus. To prevent infection 7.87%, 5.24% and 77.90% knew that abstinence, being faithful to one partner and use of condom respectively are ways of prevention.

The five STIs known by most of the respondents were; Gonorrhea 98.70%, Syphilis 72.29%, Genital warts 42.42%, Public lice 38.10% and Hepatitis 31.60%. Regarding symptoms of STIs, 90.91% knew of pain during urination, 84.45% genital discharge, 72.73% genital itching, 69.26% genital sore, 67.97% genital swelling while 69.70% knew that STIs could be asymptomatic. Less than half (45.76%) of the respondents had good knowledge regarding modes of transmission and prevention of HIV/AIDS and STIs.

With regards to prevention of HIV/AIDS and STIs, majority of the respondents used the male condom (63.14%). Among users only 9.40% were consistent users. A quarter (25.50%) of the respondents used condom at first sex and 46.31% used condom at last sex. About one-third (36.86%) of the respondents had been tested for HIV. Almost half did the test to know their status (44.95%). Majority took the test at a government health facility (60.92%). More of the respondents (62.07%) that took the test did so only once and most had it between 2009 and 2012. Amongst respondents that had been tested less than half (43.68%) did the test with their main sexual partner.

About a quarter of the respondents (28.38%) reported previous episode of an STI, among this group, the first step they took was to seek counsel from family and friends (59.70%), go to the chemist (19.40%) while 14.93% went to the health facility. More of the respondents eventually sought treatment from a traditional healer (40.30%), followed by 37.31% that got treatment at a chemist and 22.39% that got treatment at a health facility.

The study showed that a higher proportion of the young respondents less than 30years of age used condom at last sex ($p=0.006$). Age also affected HIV testing, a higher proportion of the respondents between 30-40years of age had tested ($p=0.003$) and also tested with their main partner ($p=0.001$). It also shows that respondents' level of education affected the use of condom at first sex. A higher proportion of those with a secondary/tertiary education used condom at first sex ($p=0.019$). Also, there was an association between level of education and HIV testing with main sexual partner. A higher proportion of the respondents with secondary/Tertiary education had HIV test with main partner ($p=0.006$).

There was an association between marital status and condom use. A higher proportion of single respondents used condom ($p<0.000$). It's also seen that there's an association between marital status and condom use at last sex. A higher proportion of the single respondents used condom at last sex ($p<0.000$). Marital status also affected HIV testing. More of the married respondents had done the HIV test ($p=0.015$). An association was observed between marital status of respondents and testing with main partner. More of the married respondents had HIV testing done with their

main partners (p<0.000). This study revealed that the closing time of respondents affected HIV testing. A high proportion of the respondents who closed late had done the test (p=0.030).

This study also showed that cigarette smoking and alcohol consumption affected frequency of condom use. Nonsmokers and respondents who didn't take alcohol were more likely to be consistent users (p=0.040 and p<0.000) respectively. Hemp smoking affected condom use and HIV testing, more of the respondents who smoke hemp used condom (p=0.022), whereas non Hemp smokers were more likely to have been tested for HIV (p=0.008).

There was an association between level of knowledge and HIV testing. More of the respondents with good knowledge had been tested (p=0.018). The study revealed that a higher proportion of respondents who practiced inappropriate STI treatment used condom at last sex (p=0.040).

DISCUSSION

This study was done among NURTW commercial bus drivers in Mushin Local Government Area of Lagos state. More of the respondents in this study were in the sexually active age group, fairly educated, married, resumes early at the garages and closed late. Regarding social habits, alcohol consumption (59.72%), cigarette smoking (21.37%) and hemp smoking (24.68%) was popular among this study population compared to the result of a study carried out among intercity commercial divers in Ilorin, Kwara state in 2007 where 24.6% consumed alcohol, 3.9% smoked hemp and 24.3% smoked cigarette.[7]

With regards to specific HIV/AIDS and STI preventive measures, majority of respondents (77.90%) in this study knew about condom use as a preventive measure which was high compared to what was obtained during secondary analysis of data obtained from a market survey carried out in 2008 in Lagos where only 54.30% of male respondents knew that condom can be used for prevention. As regards other preventive measures respondents knew in this study, 7.87% knew about abstinence, 5.24% being faithful to a single partner, this is very low compared to the result obtained from the same Lagos market survey mentioned above where 50.40% and 22.70% of the male respondents respectively knew that being faithful and abstinence are preventive measures.[15]

A higher proportion of the respondents in this study compared to a sample of brothel based female sex workers in Lagos knew that STI could be asymptomatic (69.70% vs 13.9%). Prophylactic use of herbal mixtures as a preventive measure was also commoner among the NURTW (3.37% vs 2.5%). Also in this study, about 4.50% of those who knew that HIV/STIs could be prevented took antibiotics as a preventive measure which was low compared to the 6.5% of the brothel based female sex workers mentioned above.[16]

Majority of the respondents (63.14%) had used the male condom in the past which is similar to the results obtained in a study carried out among long distance workers in East Africa where more than 60% of respondents used condom.[9] Hemp smokers and single men in this study were more likely to use condoms (p=0.022 and p<0.001 respectively). However, only 9.40% of the respondents who used condoms were using it consistently which was low compared to 31.6% obtained in the study mentioned East African study.[9] Drivers who did not drink alcohol or smoke cigarette were more likely to use condom consistently (p<0.001 and p=0.040 respectively). This is not surprising because alcohol consumption has been linked to risky sexual behavior from previous studies.[17]

Condom use with risky sexual partners (which includes sex workers and casual friends) was reported by about ten percent of the NURTW in this study which is lower than what was reported by the drivers who participated in the 2010 Nigerian Integrated Biological and Behavioral Surveillance Survey where 83.7% (9170/10955) of the respondents reported condom use with commercial partners and 63.9% (7001of 10955) with casual partners.[18] Condom use increased from one quarter of respondents at first sex to almost half at last sex, this is slightly low compared to the result obtained in a study carried out among automobile repair workers in Ibadan Nigeria where 59.00% used condom at their last sexual encounter.[19]

A higher proportion of respondents with a secondary/tertiary education used condom at first sex compared to those with primary or no formal education (p=0.019) Single respondents were more likely in this study to have used condom at last sex compared to the married ones p<0.001, age was also associated with condom use, higher proportion of young drivers less than thirty years of age were using condoms compared to the older ones p=0.006.

About one third of the respondents had been tested for HIV mainly to know their status which is similar to the result gotten from a study carried out among girls in some selected schools in Malawi where 31% of respondents had done HIV testing in the past and 87.1% took the test to be sure of their status.[20] Among the drivers who had been tested, almost half had done it in the preceding year which is high compared to the result of the 2008 Nigerian National Demographic and Health survey where only 7% had HIV test in the preceding year.[21]

Respondents with good knowledge of STIs and HIV were more likely to have been tested for HIV (p=0.018). Age was observed to affect HIV testing, a higher proportion of the respondents between 30-40years of age had tested (p=0.003) and also tested with their main partner (p=0.001). A higher proportion of the married respondents and those with secondary/Tertiary education had HIV test with their main sexual partner (p<0.001 and p=0.006 respectively). Drivers who did not smoke hemp and closed late at the garage were more likely to have been tested for HIV (p=0.008 and p=0.030 respectively).

The pattern of STI symptoms reported by respondents in this study was similar to the results obtained from a study done in New Delhi, India in 2007. Genital discharge was the commonest symptom followed by genital itching. However the prevalence was much lower among the Indian respondents.[22] A quarter of the respondents had an STI in the past which was high compared to the results obtained in the National HIV/AIDS and Reproductive Health Survey of 2007 where only 3.00% of the respondents reported previous history of STI.[23] About places where treatment was obtained, poor treatment seeking behavior occurred in majority of cases. Less than a quarter were treated in health facilities, more than a third by chemist while more received treatment from traditional healers. Respondents in the 2007 study mentioned above reported comparatively better treatment seeking behavior with 35.00% mentioning health facilities, 11.00% traditionalists and 13.00% chemists. The choice of treatment venue was based mainly on the cost of treatment, geographical proximity, family influence and expectation of good quality service, similar to the reasons given in this study.[22] A higher proportion of respondents who used inappropriate STI treatment measure used condom at last sex (p=0.040).

CONCLUSION

This study carried out among commercial bus drivers NURTW Mushin Lagos state revealed that less than half of the drivers had good knowledge about modes of transmission and prevention. The measures used in prevention of STI were condom and HIV test. Consistent condom use was practiced by few. Condom use with risky partner was poor while majority had not been tested for

HIV. More of the respondents who had reported prior symptoms of STI were treated by traditional healers.

Seminars and training workshops should be organized for NURTW in garages on prevention and treatment of HIV/AIDS and STI. HIV counseling and testing, STI counseling services should be provided in garages to promote access while members should also be trained as peer educators.

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Table A: Relationship between socio-demographic variables and condom use

Variable	**Condom use**		**Total**	**X^2**	**P value**
	Yes Frequency (%)	**No Frequency (%)**			
Hemp	**Ever used condom**				
Doesn't smoke	102(70.3)	72(41.4)	174		
Smokes	43(75.4)	14(24.6)	57		
Total	145(62.8)	86(37.2)	231	5.20	0.023
Marital status	**Ever used condom**				
Married	83(53.9)	71(46.1)	154		
Single	66(80.5)	16(19.5)	82		
Total	149(36.1)	87(36.9)	236	16.26	0.000
Cigarette smoking	**Consistent condom use**				
Doesn't smoke	14(12.3)	100(87.7)	114		
Smoke	0(0.0)	33(100.0)	33		
Total	14(9.5)	133(90.5)	147	4.48	0.040
Alcohol	**Consistent condom use**				
Doesn't take alcohol	13(21.3)	48(78.7)	61		
Takes alcohol	1(1.2)	84(98.8)	85		
Total	14(9.6)	132(90.4)	146	16.61	0.000
Education	**Condom use at first sex**				
Secondary/Tertiary	33(31.7)	71(68.3)	104		
No formal/Primary	5(12.5)	35(87.5)	40		
Total	38(26.4)	106(73.6)	144	5.50	0.019
STI treatment	**Condom use at last sex**				
Appropriate	2(25.0)	6(75.0)	8		
Inappropriate	25(69.4)	11(30.6)	36		
Total	27(61.4)	17(38.6)	44	0.03	0.040
Marital status	**Condom use at last sex**				
Married	29(37.7)	48(62.3)	77		
Single	49(74.2)	17(25.8)	66		
Total	78(54.5)	65(45.5)	143	19.18	0.000
Age	**Condom use at last sex**				
<30 years	39(67.2)	19(32.8)	58		
30-40 years	26(50.0)	26(50.0)	52		
>40 years	13(39.4)	20(60.6)	33		
Total	78(54.5)	65(45.5)	143	10.26	0.006

Table B: Relationship between socio-demographic variables and HIV test

Variable	Ever had HIV test		Total	X^2	P value
Age	**Yes**	**No**			
<30 years	20(24.1)	63(75.9)	83		
30-40 years	42(50.0)	42(50.0)	84		
>40 years	26(37.7)	43(62.3)	69		
Total	88(37.3)	148(62.7)	236	11.99	0.003
Marital status					
Married	66(42.9)	88(57.2)	154		
Single	22(26.8)	60(73.2)	82		
Total	88(37.3)	148(62.7)	236	5.87	0.015
Closing time					
Early (4-7pm)	25(28.4)	63(71.6)	88		
Late (After 7pm)	63(42.6)	85(57.4)	148		
Total	88(37.3)	148(62.7)	236	4.73	0.030
Knowledge of HIV/AIDS and STI					
Good	49(45.4)	59(54.6)	108		
Poor	39(30.5)	89(69.5)	128		
Total	88(37.3)	148(62.7)	236	5.56	0.018
Hemp					
Doesn't smoke	74(42.5)	100(57.5)	174		
Smokes	13(22.8)	44(77.2)	57		
Total	87(37.7)	144(62.3)	231	7.11	0.008
Education	**HIV test with main partner**				
Secondary/Tertiary	34(52.3)	31(47.7)	65		
No formal/ Primary	4(18.2)	18(81.8)	22		
Total	38(43.7)	49(56.3)	87	7.78	0.006
Age					
<30 years	5(26.3)	14(73.7)	19		
30-40 years	24(57.1)	18(42.9)	42		
>40 years	9(34.6)	17(65.4)	26		
Total	38(43.7)	49(56.3)	87	14.87	0.000
Marital status					
Married	37(56.1)	29(43.9)	66		
Single	1(4.8)	20(95.2)	21		
Total	38(43.7)	49(56.3)	87	17.04	0.000

Table C: Relationship between education and knowledge of respondents

Variable	Good Knowledge Frequency (%)	Poor Knowledge Frequency (%)	Total	X^2	P value
Level of education					
Secondary/Tertiary	85(50.0)	85(50.0)	170		
No formal/Primary	23(34.8)	43(65.2)	66		
Total	108(45.8)	128(54.2)	236	4.40	0.036

ASSOCIATION BETWEEN DOMINANT HAND GRIP STRENGTH WITH THEIR ANTHROPOMETRIC VARIABLES AMONG FEMALE LABOURERS

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ABSTRACT
Background: Physical strength can be assessed by means of hand grip strength. Correlations between physical strength and anthropometric variables have been established in female populations. Few studies confirm this correlation among female labourers. **Objectives**: The objective of this study was to determine the association between dominant hand grip strength with their anthropometric variables among female labourers. **Methods**: 60 female subjects comprising 30 female labourers working in different construction sites and 30 female non-labourers participated in this study. The dominant and non-dominant hand grip strength with their anthropometric variables: height, weight and body mass index were obtained. Pearson's product moment correlation coefficient was used to determine the relationship between the dominant hand grip strength and the anthropometric variablens individually. Level of significance was set at P=0.05. **Results**: indicate highly significant negative correlations between weight, height, body mass index and dominant hand grip strength of female labourers.
The result of this study showed that dominant hand grip strength was not closely related with the selected anthropometric variables of height and body mass index except weight among female labourers.

Keywords: *Hand grip dynamometer, Body mass index, Arm position,*

INTRODUCTION

Hand grip strength is the measurable method of applying pressure with the hand, finger, or both. The strength of hand grip is the effect of forceful flexion of all finger joints with the maximum voluntary force that the subject is capable of applying under normal biokinetic conditions (Coldiron, 2001). It is assessed by instructing an individual to forcefully squeeze, grip, or pinch a dynamometer; results are expressed in either pounds or kilogrammes of pressure. Hand grip strength because of its physiological properties is affected by a number of factors, which include age, gender and body size. Grip strength training is useful in various professions where people work with their hands (Geere *et al.,* 2007).

There has been wide medical and ergonomic research on grip strength. This has led to the generation of normative data. Average values exist for both men and women as well as for different types of grip in different positions. We discovered that grip strength either increases or decreases depending on the arm position at which the grip strength is being measured. A person's grip strength usually results in having the strongest grip strength when their arm is extended at 90^0 before their body, as opposed to the other extreme arm positions, rested at one's side or held straight up above one's head. Grip strength is not optimal if one's arm is extended backwards beyond the resting position at the body's sides. We can conclude that grip strength is affected via the different arm muscles and their ability to contract (Greere *et al*, 2007).

The grip strength was reported to be higher in dominant hand with right handed subjects, but no such significant differences between sides could be documented for left handed people (Incel *et al.,* 2002). Right and left hand grip strength was positively correlated with weight, height and body surface area (Chatterjee and Chowdhri, 1991). Grip strength was found to be a significant

determinant of bone mineral content and bone area at the forearm sites and had a positive correlation with lean body mass and physical activity. It determines the muscular strength of an individual. Grip strength serves as the most common assessment method for upper extremity muscle strength (Foo, 2007).

A reduction in grip strength is associated with decreased functional ability (Guo, 1996). The grip strength may be a useful nutritional status indicator, particularly where anthropometric measurements fail to distinguish undernourished from underweight persons. However we need to remember that grip strength measures only one dimension of functional ability (Fry *et al*, 2006). Grip strength has long been thought of as a possible predictor of overall body strength but little information is available regarding this. Hence, this study is designed to investigate the association of various anthropometric variables with handgrip strength among female labourers.

METHODS

Experimental approach

The subjects were selected using a purposive sampling technique; the research design was experimental study.

Subjects

Sixty (60) subjects (30 healthy female labourers and 30 female non- labourers) aged 18-40 years participated in the study. The female labourers were recruited from different construction sites in Lagos State and the female non-labourers were recruited from different residential areas in Lagos State metropolis, Nigeria using purposive sampling technique. Prior to the commencement of the study, ethical approval was sought and obtained from the Health Research and ethics Committee of Lagos University Teaching Hospital (LUTH). The aims and objectives of study were clearly explained to the participants as contained in the informed consent form. Only those who consented were included in the study.

Four anthropometric traits, such as height, weight, body mass index, and right and left hand grip strengths were taken for each subject. All the ages of the subjects were also recorded.

Procedure

The grip strength of both right and left hands were measured using a standard adjustable digital hand grip dynamometer in standing position. The subject holds the dynamometer in the hand to be tested, with shoulder adducted, arm at right angle and the elbow in 90 degree flexion. The handle of the dynamometer was adjusted when required. The base rest on first metacarpal (heel of palm), the subjects squeezes the dynamometer with maximum force, which was maintained for about 5 seconds three times. The average was recorded in kilograms.

Statistical Analysis

Data was analyzed using Statistical Package for Social Science version (SPSS) 17 and summarized with descriptive statistics of mean and standard deviation. Student paired t-test was applied for the comparison of all the variables between female labourers and controls. Pearson's correlation coefficient was applied to establish the correlation of dominant hand grip strength with other variables among female labourers. Linear regression analyses were carried out with hand grip strength separately as a dependent variable and the rest of the anthropometric variables as independent. Level of significance was set at $p<0.05$.

RESULTS

The age of the subjects ranged from 18-40 years with a mean age of 27.08 years. The mean weight for all the participants was 60.78 kg, with the weight ranging from 35- 85 kg. The height of the

subjects ranged from 142-171 cm with a mean value of 157cm. The mean Body mass index (BMI) was 24.76kg/.m^2.

Correlation coefficient(r) between female labourers and female non-labourer dominant hand grip strength and each anthropometric variable.

Table1 showed correlation between the dominant hand grip strength of female labourers and each of the variables, Age (r = -0.068, p = 0.721), Height (r = -0.134, p = 0.480), weight (r = -0.231, p = 0.219), BMI (r = -0.144, p = 0.449). The anthropometric variables had strong negative correlations with the dominant hand grip strength. Also, the correlation between the dominant hand grip strength of non-female labourers and each of the variables. Age (r = - 0.226, p = 0.230), Height (r = 0.299, p = 0.109), weight (r = 0.004, p = 0.739) and the BMI (r = -0.119, p = 0.533) showed that the anthropometric variables had both negative and positive correlation with the dominant hand grip strength..

Table 2: showed that Non-female labourers have higher mean value in all the anthropometrics variable assessed i.e. Height (1.57m), Weight (63.93Kg), BMI (25.96Kg/M^2), than the female labourers with height (1.57m), weight (57.60Kg), and BMI (23.57Kg/M^2) respectively.

Table 2 also showed that the Non-dominant handgrip strength (23.01Kg) of the non-female labourers is higher than the female labourers (20.98Kg), while the Dominant hand grip strength is higher in the female labourers (26.73Kg) than the non-female labourers (23.81Kg).

DISCUSSION

The purpose of this study was to investigate the correlation between dominant hand grip strength and some anthropometric variables such as weight, height, body mass index of female labourers and non-female labourers. 60 subjects (30 labourers and 30 non- labourers) participated in this study.

The result revealed that the anthropometric variables (age, height, weight and BMI) of female labourers had negative correlations with the dominant hand grip strength. This finding is contrary to the results of the study of Oseloka *et al*. (2006) that reported a positive correlation of hand grip strength with all anthropometric variables associated with Body mass index. It also disagrees with the findings of the study of Chandransekeran *et al.* (2010) and Koley *et al.* (2009) who in their own study found a positive correlation between age, height, weight and BMI. The findings of the present study indicate high significant difference in hand grip strength and weight between female labourer and non-female labourer. This result supports the findings of the study of Koley, *et al.* (2009) who reported a statistically significant difference in weight and hand grip strength of the female labourers studied in their own study.

Grip strength has long been thought of as a possible predictor of overall body strength. But little information is existing regarding this. Smith *et al*. (2005) found a direct correlation in grip strength and general body strength in elderly female populations. Fry *et al*. (2006) also found a correlation between grip strength and performance in American junior male weightlifters.

It is also reported that hand grip strength determines the muscular strength of an individual (Foo 2007). Findings from the present study indicate that female labourers have lower mean value of weight and BMI and non-dominant handgrip as compared to non female labourer. This result agrees with the findings of the study of Chilima and Ismail. (2001) and Koley *et al*. (2009) who confirmed that those labourer with lower BMI category had lower mean hand grip strength, while the study of Pieterse *et al.* (2002) reported that low nutritional status defined by low BMI emerged as a significant determinant of impaired hand grip strength.

In fact, Chilima and Ismail 2001 reported that hand grip strength was positively associated with nutritional status, including health status and socioeconomic conditions. Nevertheless, females working in different construction sites have poor nutritional status due to their low

socioeconomic conditions but they require more physical strength i.e hand grip strength to perform their daily work efficiently, obviously poor nutritional status fails to provide adequate hand grip strength to them and as result affect their productivity. The result of this study shows that dominant hand grip strength was not closely related with the selected anthropometric variables of height and body mass index except weight in non–female labourer. Therefore, the higher the body weight, the greater the grip strength and the higher the ability to regain functional grip strength.

Practical Application

Close monitoring of anthropometric variables should be instituted during hand rehabilitation to augment other treatment procedures and to plan effective preventive strategies to avoid their various complications.

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Table 1: Correlation coefficient (r) between female labourers and female non-labourers dominant hand grip strength and each anthropometric variable.

	Labourers		Non-labourers	
	r	P-value	r	P-value
Age (years)	-0.068	0.721	- 0.226	0.230
Weight (Kg)	-0.231	0.219	0.004	0.739
Height (M)	-0.134	0.480	0.299	0.109
BMI (Kg/M^2)	-0.144	0.449	-0.119	0.533

Key:
r = Pearson product-Moment - correlation coefficient

Table 2: Comparison of Hand Grip Strength and Anthropometric variables between the Female Labourers and Female Non- Labourers

Table 2: Comparison of Hand Grip Strength and Anthropometric variables between the Female Labourers and Female Non- Labourers					
	Labourer	Non-labourer			
Significance	Mean+ SD	Mean+ SD	t-test	P-value	
Weight (Kg)	57.60+ 8.23	63.93+11.3	2.493	0.016	S
Height (M)	1.57 + 0.08	1.57+0.06	0.120	0.905	NS
BMI (Kg/M^2)	23.57 + 4.31	25.96+4.99	1.983	0.052	NS
D H G (Kg)	26.73+ 28.39	23.81+5.1	-0.556	0.580	NS
N D H G (Kg)	20.98+ 5.33	23.01+5.33	1.450	0.152	NS

KEY:
NS: Not-significant
S: Significant
DHG: Dominant Hand Grip
NDHG: Non- Dominant Hand Grip

PROTECTING THE HEALTH OF NIGERIAN FOOTBALLERS: DESIGN AND APPLICATION OF A LOW-COST PRE-PARTICIPATION SCREENING PROGRAMME (SAFEPLAY)

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ABSTRACT
Background: Practicable injury prevention and health protection strategies are necessary to keep players in action in developing countries especially at the grassroots and youth football levels where medical attention is almost absent. Pre-participation screening of players is a viable option. However, this is not a common practise in Nigeria because of funds and lack of implementation framework. **Objective:** To design a low-cost pre-participation screening (PPS) programme tagged "SafePlay" that would be culturally relevant at all levels of participation in Nigerian football. The study further analysed the findings and consequences of the SafePlay PPS on a cohort of sub-elite football players. **Methods:** A cohort study involving 706 sub-elite male youth football players (from 36 teams) was conducted. A framework and culturally adapted questionnaire for the SafePlay PPS programme modelled after the FIFA's Medical Assessment and Research Centre, American Heart Association, American College of Sports Medicine and the European PPS protocols was developed. This was administered by 6 assessors on the aforementioned cohort of players prior to the 2012/2013 league season. The PPS programme evaluated specific aspects of players' football and medical history, family and social history and physical examination and fitness. **Results:** All the SafePlay PPS questionnaires were adequately filled and returned by assessors. Of the total cohort of players (17.67 ± 1.11 years; range: 14 – 19 years), 25 (3.5%) players had moderate to severe injuries that would temporarily restrict them from immediately participating in league matches but no player was eventually restricted to participate based on musculoskeletal problems. Moreover, 17 (2.4%) players were temporarily refused participation based on cardiovascular risk factor assessment; 2 (0.3%) of which were recommended for restriction of play due to subsequent findings from additional comprehensive investigations. None of the players had a sub-optimal physical fitness score that resulted in restriction. **Conclusion:** A low-cost PPS programme tagged SafePlay was successfully applied to a cohort of football players. Implementation of a culturally adapted PPS programme such as the SafePlay is feasible in Nigerian Football. A nationwide implementation by the Nigeria Football Federation and other stakeholders is recommended to ensure safety among players participating in football leagues and tournaments across the country.

Keywords: *Injury Prevention, Sudden Death, Pre-Participation Screening, Sports.*

INTRODUCTION

Football players are known to suffer relatively high rates of injuries compared to participants in other sports and occupations (Hawkins and Fuller, 1999; Kirkendal *et al.*, 2010). Apart from the risk of sustaining musculoskeletal (MS) injuries while playing football, athletes are also predisposed to having a sudden cardiac arrest which may eventually result in a sudden cardiac death (SCD). Most of the deaths in athletes are due to disorders of the cardiovascular (CV) system (Harmon *et al.*, 2012).

Football players in Nigeria are faced with huge challenges. A major one is the problem of low financial resources for professional, amateur and youth clubs; as most of them are not well funded. The state of playing surfaces and appropriate equipment are not of good quality despite their important role in injury prevention. Other problems faced by these players include low remuneration and little to no medical support as most clubs do not have qualified medical personnel (Owoeye *et al.,* 2013). Primary prevention of MS injuries and CV events among players is therefore crucial in this region of the world.

Thus, the preventive detection of 'subjects at risk' has been recognised a priority and a pre-participation screening (PPS) has been established to be an important step towards the prevention of MS injuries and SCD even among apparently healthy competitive athletes (Corrado *et al.,* 2005; Thu¨nenko¨ tter *et al.,* 2006; Maron *et al.,* 2007; Ljungqvist *et al.,* 2009). A list of CV risk factors that can be obtained directly from athletes through a history-based assessment and simple examination procedures have been identified (JN7, 2003; Maron *et al.,* 2007). Such factors include family history of CV disease, elevated blood pressure, body composition, age and smoking (JN7, 2003; Maron *et al.,* 2007; ACSM, 2009).

Pre-participation screening programmes are used by most sport governing bodies to evaluate eligibility for competitive sports in athletes. The major objective of a PPS is to identify athletes at MS and CV risks; in order to protect athletes' health. The international governing body for football (FIFA) has mandated PPS as an obligatory procedure for all member associations at all levels of participation. However, organised football leagues and administrations in Nigeria currently do not practise PPS partly owing to poor funding.

The objective of this study was to design and implement a low-cost PPS programme tagged "SafePlay" that would be culturally applicable at all levels of participation in Nigerian football. The study further analysed the findings and consequences of the PPS on players.

METHODS

Description of the SafePlay PPS programme

The SafePlay PPS programme (Figure 1) was modelled after the FIFA's Medical Assessment and Research Centre (F-MARC, 2013), American Heart Association (Maron *et al.,* 2007) American College of Sports Medicine (ACSM, 2009) and the European (Corrado *et al.,* 2005) PPS protocols. A culturally adapted SafePlay PPS questionnaire (Appendix) was also generated from these protocols for data documentation. The PPS is mainly based on players' football history, medical history, family history and selected physical examinations vis-a-vis MS and CV risk assessments, body composition and an explosive leg strength test for physical performance assessment.

Administration

Prior to the commencement of administration of the SafePlay PPS programme, 6 assessors - 4 physiotherapists (1 for each station and the pre-assessment station) and 2 assistants (final year physiotherapy students) were invited to a 2-hour workshop on the SafePlay PPS programme. In the process, all the instruments and procedures that were used for the study were pre-tested and assessors got acquainted with the screening protocols.

The SafePlay PPS was administered on 706 sub-elite football players (from 36 teams made up of 20 Premier League teams and 16 League I teams) of the Lagos Junior League prior to the 2012/2013 league season on a team-by-team basis. Each player went through a pre-assessment station and then through 3 assessment stations (Figure 1) and assessment scores were documented on the PPS questionnaire (Appendix). Players responded and were examined as

necessary based on questions specified on the questionnaire (Appendix). Furthermore, players' BP, body composition and physical performance parameters were measured and documented in the questionnaire. Players' physical performance as characterised by explosive leg strength was classified based on the reference data in the same cohort as further analysed by Owoeye et al., (2014). Players with values $\leq 25^{th}$ percentile distributions were regarded as having poor leg strength (ie $\leq$ 21cm of vertical jump). Recommendations were made for players' eligibility based on the guidelines on the last page of the SafePlay PPS questionnaire. Questionnaires were adequately filled and returned without problems.

RESULTS

Descriptive characteristics of players are as presented in Table 1. The mean age of players was 17.67 ± 1.11 years (range = 14 – 19 years). Fifty-eight (8.3%) players reported currently having at least 1 MS injury. However on physical examination, only 25 (3.5%) players had moderate to severe injuries that would temporarily restrict them from immediately participating in league matches. They were advised on appropriate interventions (Figure 2) and educated on likely time of return to play. No player was eventually restricted to participate based on MS problems. Conversely, 17 (2.4%) players were temprarily refused participation due to deductions from their CV risk stratification (15 had moderate while 2 had high risk – based on ACSM's CV risk stratification). These players were referred for treatment and further inveatigations (Figure 2).
Subsequent findings from ECG along with other successive assessments resulted in recommending the restriction of 2 players from participating in the 2012/2013 league season. None of the players had a sub-optimal body composition value using internationalclassification guidelines. A total of 197 (28%) players had poor explosive leg strength. They were advised and instructed on performance related fitness. However, 2 (0.3%) players with the least leg strength were recommended for temporary restriction and further MS assessment and interventions (Figure 2).

DISCUSSION

This study explored the feasibility of a PPS in a cohort of youth football players in Lagos, Nigeria. The high level of compliance and results obtained from the various assessments demonstrates that the SafePlay PPS has the potentials for implementation. Overview of returend copies of the PPS questionnaire and feedbacks from assessors revealed that the completion of the SafePlay questionnaires showed no fundamental problems. Forty-four players needed further further MS and CV interventions before recommendations were made. However, only a few of these players needed further expensive and elaborate investigations such as X-rays, ECG, expert consultation etc. This suggests the economic suitability and practicability of the SafePlay PPS programme; only high risk players needed further comprehensive assessements.

Most of the studies reporting regular PPS focus mainly on the CV system since prevention of SCD in sports is a primary and essential goal (Rao *et al.*, 2010; Behera *et al.*, 2011). The results of the present study in male youth footballers show that MS findings with the need of either therapeutic or preventive implication are rather frequent and have to be taken into account. Results show that recommendations for therapy and prevention can be based mainly on clinical findings. In most of the MS cases, either physiotherapeutic measures or the improvement of MS fitness by strength or sensory motor training was advised. This approach is supported by previous studies (Abernethy and Bleakley, 2007; Mayer *et al.*, 2012).

The explosive leg strength of a football player indicates his leg muscle power. This is a very important lower extremity parameter that implicates a player's level of performance and suceptibility to injuries. A total of 197 (28%) players were reported to have poor explosive leg

strength. Categorisation of players' explosive leg strength was based on the percentile distribuion of the cohort of players. Male youth football players with a vertical jump height of ≤ 21cm; that is ≤ 25% percentile rank compared to peers was regarded as poor while those > 26% through 100% percentile rank compared to peers were classified to range through fair, good and excellent (Owoeye et al, 2014). They were educated on the risks associated with poor physical performance rating and advised on performance related fitness training to improve their leg strength before the commencement of the league season. Nevertheless, 2 (0.3%) players with very poor leg strength (the least values) were recommended for temporary restriction and further MS assessment and interventions.

The SafePlay PPS programme includes MS, CV risk and physical performance assessment. However, it has an enormous consideration for CV risk assessment in players because of the grave consequences associated with sudden cardiac attack. A small, but notable proportion of players have been reported to die suddenly while participating in vigorous sports (Maron, 2003; Weiler *et al.*, 2012). Sudden cardiac death in football players is a global problem and Nigeria is not exempted as a risk nation. A number of Nigerian footballers (mostly youth) have been reported to have died while playing football in Nigeria. A few of affected players who died during training or competitive football in Nigeria include: Samuel Okwaraji (24 years; National Stadium, Lagos), Amir Angwe (29 years; Onikan Stadium, Lagos), Tunde Charity (19 years; Benin City, {during a Nigerian Premier League match}), Emmanuel Ogoli (21 years; Samson Siasia Stadium, Yenogoa; {during a Nigerian Professional League match}), Niyi Lawal (26 years; Odogbolu LGA, Ogun State; grass-root) (Nigeria News Online, 2013).

Early detection of clinically significant CV disease through pre-tournament or pre-participation screening protocols has been proven to permit timely therapeutic interventions that may alter clinical course, prevent sudden death on the field of play and significantly prolong life (Corrado *et al.*, 2005). However, PPS is not a common practise in Nigerian football. The logical reason for the unpopular practise of PPS in Nigeria may be because of cost and lack of expertise. Resources are not available to comprehensively evaluate athletes before participation in competitive sports as advised by FIFA (F-MARC, 2013) and the IOC (Ljungqvist *et al.*, 2009). Therefore, detailed and comprehensive CV evaluation of players need to focus on individuals who are at greatest possible risk subsequent to the the first-line assessment as illustrated in the SafePlay PPS protocol. This study has provided a conceptual design for a PPS programme that is practicable for implementation in Nigerian football.

CONCLUSION

A low-cost PPS programme tagged SafePlay culturally designed for Nigerian football was successfully applied in the cohort of football players studied. Eligibility refusals were rare. However, recommendations for treatment and prevention were frequent and referral for comprehensive investigations were minimal. Implementation of a culturally adapted PPS programme is feasible in Nigerian Football. The SafePlay PPS programme has the potential to serve as an economically viable and practicable screening tool among Nigerian footballers.

RECOMMENDATION

Pre-participation screening programmes should be a mandatory pre-tournament and pre-season procedure for Nigerian football. We recommend a nationwide implementation of the SafePlay PPS programme by the Nigeria Football Federation and other stakeholders in order to ensure safety among players participating in football leagues and tournaments across the country. Although the SafePlay PPS programme is specially designed for implementation in football, its adaptation in other vigorous sports is also encouraged.

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Table 1: Descriptive Characteristics of Players

	Premier League (n = 416)	League I (n = 290)	Overall (N = 706)
Physical Characteristics (Mean ± SD)			
Age (year)	17.64 ± 1.07	17.70 ± 1.17	17.67 ± 1.11
Height (m)	1.72 ± 0.06	1.71 ± 0.06	1.72 ± 0.06
Weight (kg)	63.5 ± 6.78	62.65 ± 6.22	63.16 ± 6.57
Playing Position [n(%)]			
Goalkeepers	38 (9.3)	25 (8.7)	63 (9.0)
Defenders	126 (30.9)	91 (31.5)	217 (31.1)
Midfielders	136 (33.3)	97 (33.6)	233 (33.4)
Strikers	108 (26.5)	76 (26.3)	184 (26.4)
Total	408	289	*697
Limb Dominance [n(%)]			
Right	272 (67.3)	189 (66.3)	461 (66.9)
Left	61 (15.1)	34 (11.9)	95 (13.8)
Both	71 (17.6)	62 (21.8)	133 (19.3)
Total	404	285	**689

*Incomplete data for 9 players

** Incomplete data for 17 players

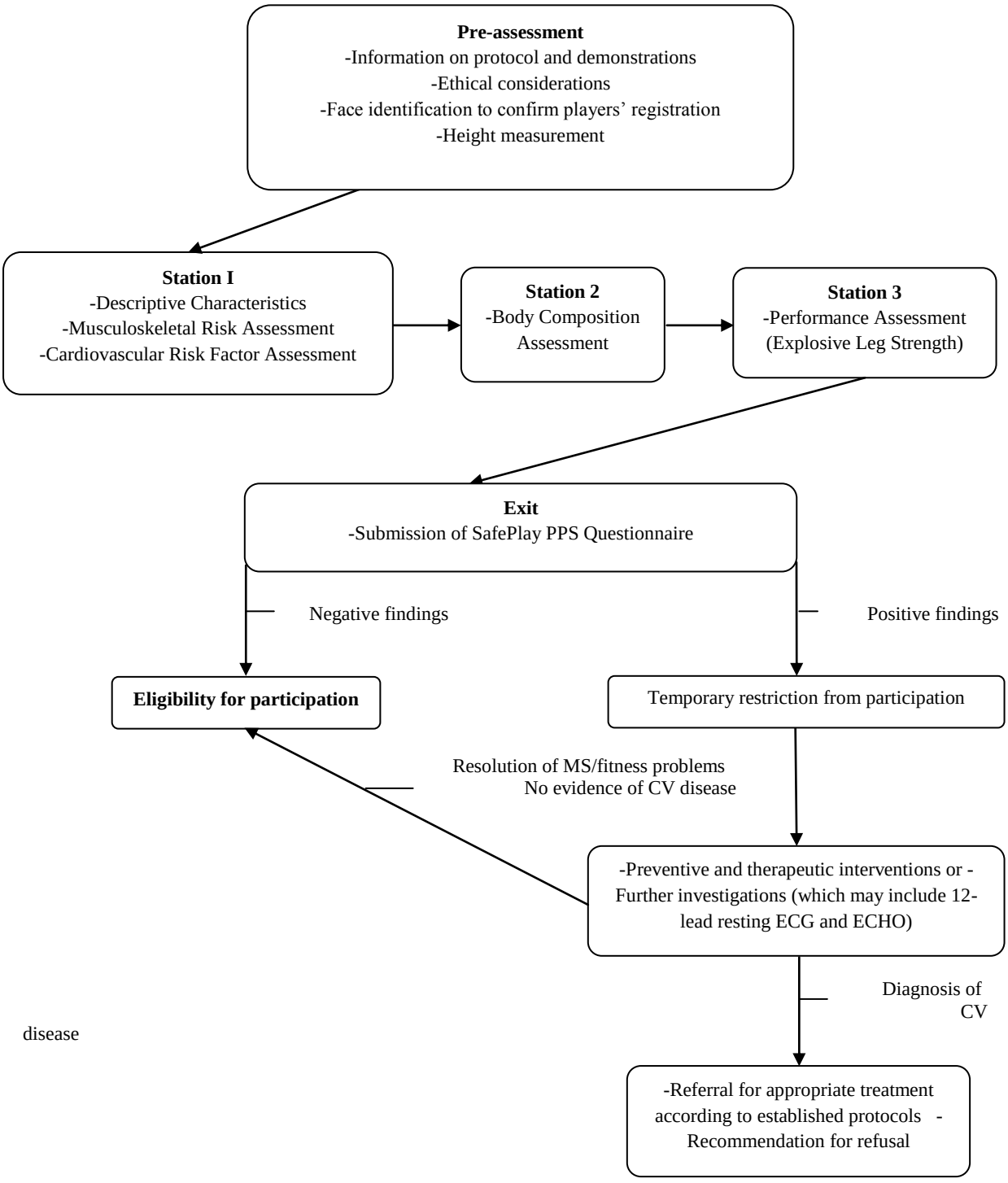

Figure 1: Flow-chart of the SafePlay PPS Protocol.

First-line screening includes stations 1 – 3 only. Players recognised to be affected by CV conditions potentially responsible for sudden death in association with sport participation are managed accordingly and restricted from participation.

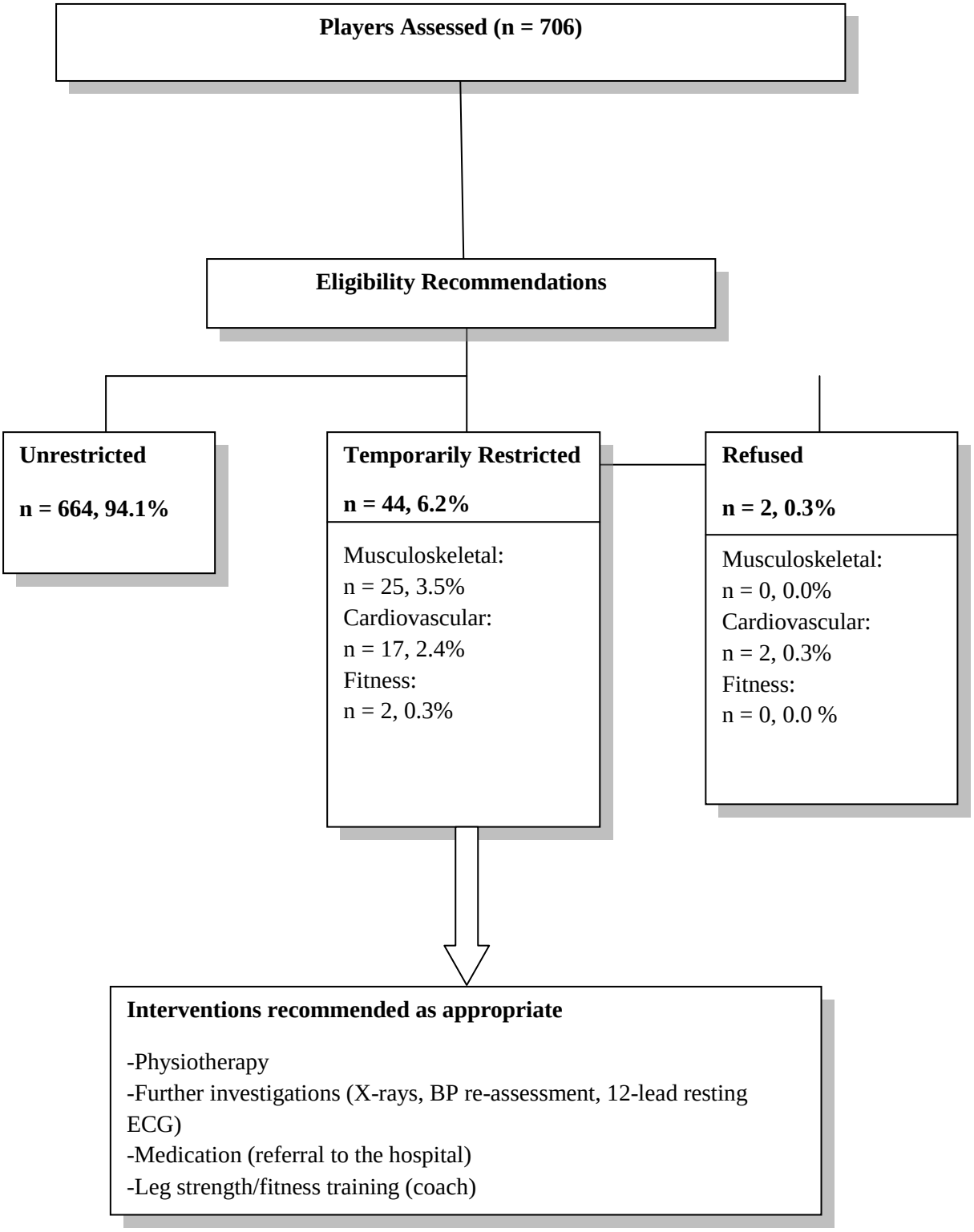

Figure 2: Findings and Deduced Consequences from SafePlay PPS Programme

EFFECTS OF AEROBIC EXERCISE PROGRAMME ON SELECTED CARDIOVASCULAR PARAMETERS, BODY COMPOSITION AND QUALITY OF LIFE OF PEOPLE LIVING WITH HIV/AIDS

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ABSTRACT
Background/Purpose: People living with HIV/AIDS who are undergoing Highly Active Antiretroviral Therapy (HAART) present with impaired functional capacity and metabolic disturbances which affect their quality of life (QoL)**.** This study was designed to evaluate the effects of aerobic exercise programme on the cardiovascular parameters, body composition and QoL of people living with HIV/AIDS. **Method:** A total of 52 patients recruited from the HIV/AIDS Prevention and Intervention Initiative (APIN) Clinic, Lagos University Teaching Hospital, Idi-Araba, Lagos participated in the study. They were randomly allocated into two groups (exercise and control groups) using the fish bowl method. Participants in the exercise group received a monthly nutritional counseling and aerobic exercise programme 3 times a week for a period of 8 weeks, while those in the control group received monthly counseling on the importance of physical activity such as walking and running. Cardiovascular parameters, body composition and QoL were evaluated at baseline and at the end of the 8th week. Descriptive statistics of mean, standard deviation and percentages was used to present the demographic data while inferential statistics of *t* – test was used to analyze the data. Level of significance was $p \leq 0.05$ **Results:** There was a significant change ($p<0.05$) in cardiovascular parameters (systolic and diastolic blood pressure, resting heart rate and VO_2max) in the exercise group. The changes in cardiovascular parameters in the control group was not significant ($p>0.05$). There was a significant reduction in the values of the body composition variables (body mass index, body fat, visceral fat, waist circumference) following the eight-week exercise programme in the study group ($p <0.05$). All domains of QoL had a significant improvement in both groups ($p < 0.05$). **Conclusion**: An 8-week aerobic exercise programme coupled with nutritional guidance was able to significantly improve cardiovascular fitness, body composition and QoL in people living with HIV/AIDS.

Keywords*: HIV/AIDS, Aerobic Exercise, Cardiovascular Parameters, Body Composition, Quality of Life*

INTRODUCTION

People living with Human Immune Virus (HIV)/ Acquired Immunodeficiency Syndrome (AIDS) undergoing Highly Active Antiretroviral Therapy (HAART) often present with problems associated with HIV infection and its therapy (Ogalha *et al*, 2011). These include metabolic changes, body and metabolic modifications, the stigma often associated with AIDS, chronic use of antiretroviral (ARV), and fear of imminent death. These problems taken together can affect the physical, social, and psychologic health components, causing a negative impact on their QoL (Ogalha *et al*, 2011). The large number of patients with HIV/AIDS put increasing demand on the health care system. Hence, there is a need to place greater emphasis on maximizing both the patient's independence and the disability and also to increase the patient's functional status so as to improve their QoL (Anderson, 2006). Exercise is consistently listed among the three most common complementary and alternative therapies utilized by HIV-infected persons to improve their QoL (Greene *et al*, 1999; Sparber *et al*, 2000; Standish *et al*, 2001; Cade *et al*, 2004), and

therapeutic exercise among people with HIV has been shown to be both beneficial and safe (Ciccolo *et al,* 2004).

Since the introduction of HAART, AIDS has become a chronic disease, enabling the use of non-pharmacological approaches such as physical exercises (Scevola *et al,* 2003; O'Brien *et al,* 2004) which maintains functionality and QoL for several years (Gomes *et al,* 2010). Therefore, maintaining the physical and functional fitness of patients with HIV/AIDS has become one of the most important therapeutic targets, particularly in the case of "wasting syndrome", which is an important loss of muscle mass (Souza *et al,* 2008).

Although life expectancy has increased, psychological problems seem to affect sero-positive subjects and their QoL (Ciccolo *et al,* 2004). There is evidence that mental health may improve by the regular practice of physical activities (Gomes *et al,* 2010). Thus, the regular practice of exercises is an interesting approach to deal with the psychological problems related to HIV infection (Gomes *et al,* 2010). Some studies have shown that subjects enrolled in programmes of aerobic or strength training may present improved emotional, mental and physical well-being (Lox *et al,* 1995) and reduction of depressive symptoms (Neidig *et al,* 2003).

People living with HIV have abnormally low functional capacities, expressed as lowered capacity to utilize oxygen and perform physical work (Keyser *et al,* 2000). Tests of an individual's ability to use oxygen, functional aerobic capacity (FAC), have shown that those infected with HIV have VO_2 max of 24% - 44% below their age-predicted normal values (MacArthur *et al,* 1993; Lox *et al,* 1995). While it is likely that the aetiology of this reduced work capacity is multi-factorial, the sparse evidence available suggests that moderate to high intensity aerobic exercise training is effective in improving the FAC of HIV-positive persons (Hand *et al,* 2008).

Hypertension is found in about 25% of people with HIV because of sedentary lifestyles and other increased cardiovascular risk factors (Malita *et al,* 2005; Blanco *et al,* 2010). The current recommendation for treating hypertension in the population with HIV is the same as in the general population: lifestyle changes, including regular exercise, are recommended with the possible addition of anti-hypertensive drugs if needed (Blanco *et al,* 2010).

Unfortunately, treating HIV with HAART results in a number of physical and psychological adverse effects. Common physical adverse effects include disorders of the nervous system (headache, pain/neuropathy, and fatigue), gastrointestinal tract (nausea, vomiting, diarrhoea), integumentary system (rash, dry skin), metabolic processes (glucose, lipid alterations, bone disease) and morphology (lipodystrophy, and lipidatrophy) (Hicks *et al,* 2003). The psychological responses may include agitation, confusion, anxiety, nightmares, hallucinations, mania and depression (Horwath, 2002). Although HAART allows for a reduction in HIV-related mortality, the extension in a patient's length of life can be associated with a reduction in their QoL (Ciccolo *et al,* 2004). As such, it is increasingly more important to develop interventions for this population that are designed to enhance their QoL. Hence, this study was designed to evaluate the effects of an aerobic exercise program on the cardiovascular parameters, body composition and QoL of people living with HIV.

METHODS

Participants

The study commenced with a total of 79 individuals living with HIV/AIDS and attending the AIDS Prevention and Intervention Initiative (APIN) Clinic of Lagos University Teaching Hospital (LUTH) who were approached for participation in the study. Nine (9) of them declined while 10 did not meet the inclusion criteria. The remaining 60 were randomly assigned into 2 groups of 30 participants each using the fish bowl method. Five participants dropped out of the exercise group without reaching 70% of completion. Three participants in the control group also

dropped out. Figure 1 shows the recruitment and allocation of the participants. Inclusion criteria were: subjects on current use of Active Retroviral drugs, age equal to or older than 18 years and availability to attend the study activities. Exclusion criteria were: pregnant subjects, active opportunistic infections, contraindication to exercise testing and training, significant cognitive impairment or inability to follow instructions, involvement in a regular exercise programme (defined as two or more structured exercise sessions weekly for six or more months prior to enrolment).

Participants in the exercise group went through an exercise training programme for eight weeks while those in the control group were given monthly counseling on the importance of physical activity such as walking and running.

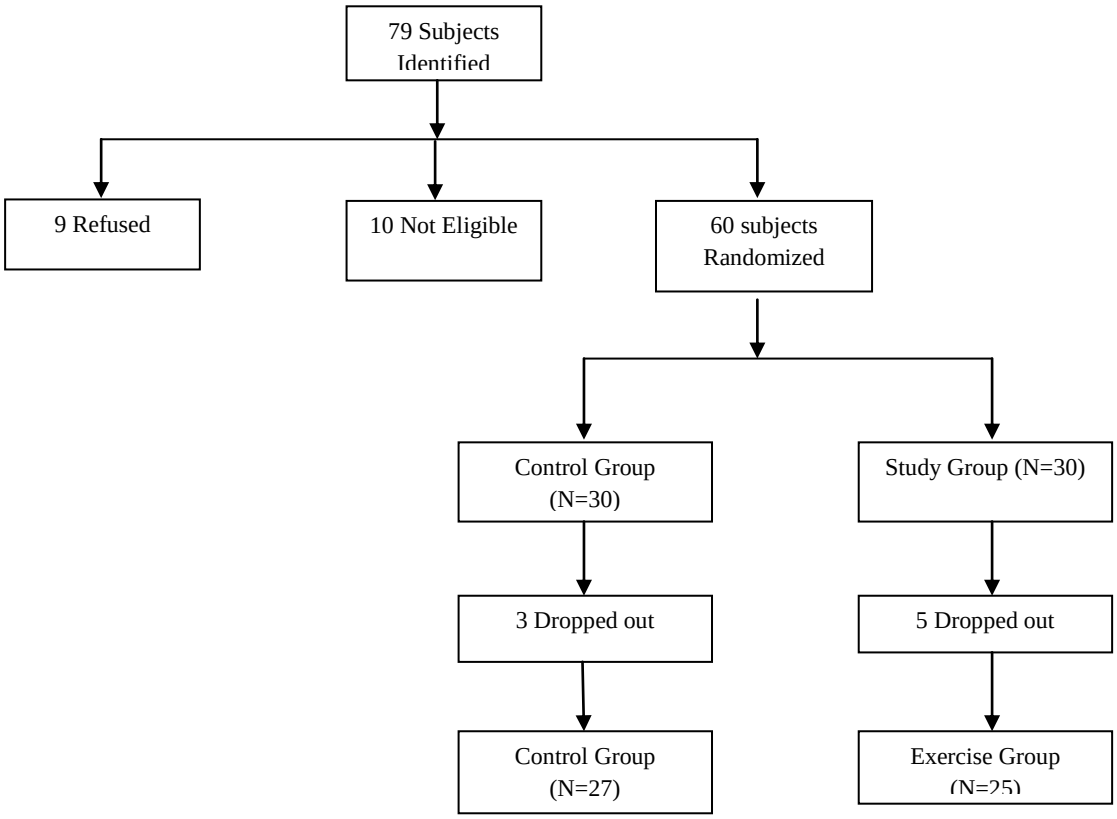

Figure 1: Recruitment and allocation of participants

Research design

The study was a randomized controlled trial.

Research Protocol

The study protocol was approved by the Research and Ethics Committee of Lagos University Teaching Hospital (LUTH) Idi-Araba, Lagos State, Nigeria (Ref.: ADM/DCST/HREC/VOL.XVI/APP/452). Informed consent was also obtained from the participants prior to participation.

Body composition: Height was measured in meters (m) using a stadiometer with participants barefooted. Weight (kg) was measured using OMRON BF511, with the subject stepping on the main unit barefooted and placing the feet on the foot electrodes with the weight evenly distributed in an upright position. Body Mass Index was measured with OMRON BF511 which uses the height information stored in the personal profile or when entering the guest mode and weight measurement to calculate the BMI (kg/m^2). The device uses the bioelectrical impedance

(BI) to estimate the body fat percentage and visceral fat percentage. In order for the scale to determine body composition, it uses the electrical impedance, along with height, weight, age and gender information to generate results based on OMRON's data of body composition. The device also calculates the body skeletal muscle mass. A flexible tape measure was used to measure waist circumference (in centimeters) at the level of the navel at the end of gentle expiration, with the subject standing upright (Jackson and Pollock, 1978). The values were taken at baseline and after 8 weeks.

Exercise training.

Participants were screened for cardiovascular risk factors. This was followed by the baseline assessment of blood pressure and heart rate. Participants in the exercise group performed aerobic exercise training on a motor-driven treadmill. Exercise was performed 3 times a week at a moderate intensity level for a maximum period of 45 minutes. Exercise was started with initial brisk walking pace, ranging from 1.5 - 2.0 mph at a 0% grade for 4 minutes to elicit a heart rate within 40-50% of age predicted maximum (220-Age) (Nieman, 2011). This was followed by a second 4-minute stage in which the speed remains the same, but the treadmill was raised to a 5% grade. The speed was increased as the training progresses until the target heart rate 75% of the predicted maximum was reached. Exercise was monitored using Borg's Rate of Perceived Exertion scale, where participants were asked to rate their perceived exertion (Borg, 1982). The blood pressure and heart rate were measured every 3 minutes as an additional monitor. The VO_2max was calculated using the equation:

VO_2max = 15.1 + (21.8 x Speed) - (0.327 x Heart Rate) - (0.263 x Speed x Age) + (0.00504 x Heart Rate x Age) + (5.98 x Gender) where speed is treadmill speed in mph; gender = 0 for females, 1 for males (Ebbeling *et* al, 1991; ACSM, 2010). All the parameters were measured at baseline and at the end of the 8th week.

Quality of life

The domains of quality of life were measured using the HIV-MOS Health Survey at baseline and at the end of the 8th week. The subscales were scored as a summated rating on a standardized 0 – 100 scale.

Data Analysis

Data analysis was done with the use of the Statistical Package for Social Sciences (SPSS) version 17.0 (SPSS Inc., Chicago, IL, USA). Unpaired *t*-test was used to determine the significance of the mean differences of variables between the groups ($p<0.05$).

RESULTS

Socio-demographic characteristics of the participants

The mean age of the participants was 40.9±8 years and 41±7.8 years for the control and exercise groups respectively. The control group was made up of 11 (39.3%) males and 16 (60.7%) females; the exercise group had 9 (30%) males and 16 (70%) females. In the control group 18 (66%) had secondary school education and 9 (36%) had tertiary education. In the exercise group, 17 (73.3%) had secondary school education and 8 (26.7%) had tertiary education (Table 1).

Changes in cardiovascular parameters

There were no significant differences in cardiovascular parameters of the participants at baseline ($p>0.05$). After eight weeks of aerobic exercise programme for the participants in the exercise group, significant differences were observed between the groups in the cardiovascular parameters monitored i.e. systolic blood pressure (SBP), diastolic blood pressure (DBP), resting heart rate (RHR) and maximal oxygen consumption (VO_2max) ($p<0.001$) (Table 2). Within

group comparison showed significant differences in the cardiovascular parameters ($p<0.001$) only in the exercise group at the end of the study (Table 3).

Changes in body composition parameters

Table 4 shows that there were no significant differences ($p>0.05$) in the body composition parameters at baseline between the groups, except for visceral fat (VF). Post intervention comparison shows that significant differences ($p<0.05$) exist in all the parameters. Also, there were no significant differences ($p>0.05$) in the within group comparison of the control group for all parameters, except for visceral fat. At the end of the study period, however, the exercise group recorded significant differences ($p<0.05$) in all the parameters except for visceral fat (Table 5).

Changes in quality of life domain

There were no significant differences in the measured QoL domains between the two groups at the beginning of the study. Significant differences were observed in all the domains between the groups at the end of the study period (Table 6). As far as within group comparisons are concerned, significant differences were observed in the measured QoL domains in the two groups at the end of eight weeks' study period.

DISCUSSION

The objective of this study was to determine the effects of aerobic exercise program on some selected cardiovascular variables, body composition and QoL of people living with HIV/AIDS. Sixty participants were selected and randomized into two groups (exercise and control groups, with 30 in each group). Five participants dropped out of the exercise group without reaching 70% of completion while 3 participants dropped out of the control group. A drop-out rate of 16% was observed in this study which is less than the rate reported in a meta-analysis on aerobic exercise and HIV/AIDS by O'Brien *et al*, (2004), in which six studies reported drop-out rates higher than 20% and two others higher than 50%. The participants in this study also achieved higher completion rates (80%) than what was reported for similar exercise intervention trials (70%, 77% respectively) by Roubenoff *et al*, (2002) and Hand *et al*, (2008) and less than the 81% reported by two other similar studies (Fillipas *et al*, 2006; Tiozzo, 2011).

There was no significant difference in all the measured variables at baseline between the two groups indicating the homogeneity of the groups. There was a reduction in the cardiovascular variables assessed in both groups following the eight weeks of the study. However, the reduction was only significant in the study group. This finding is in agreement with the report of Cicolo et al., (2004) and Ogalha *et al*., (2011) who also reported a significant reduction in the cardiovascular variables following aerobic and resistance exercise.

Furthermore, cardiorespiratory fitness (VO_{2max}) improved significantly in the exercise group compared with the control group. VO_2max remained the same in the control group despite the continued HAART treatment. This is similar to the findings of Mutimura et al. (2008), who also reported a significant improvement in the VO_2max of the participants in the intervention group compared with those in the control group. The improved VO_2 max, is an important measure of aerobic capacity related to health and longevity (Manson *et al*, 1999; Myers *et al*, 2002). HIV individuals may have up to 9% lower VO_2max values, compared with age-matched healthy individuals (Johnson *et al*, 1990). The result is also in agreement with the findings of Tiozzo (2011) that a 3-month combined moderate intensity exercise programme achieved a significant improvement in estimated VO_2 max in the exercise group while the control group showed no significant change. In contrast to the above results, others have found non-significant increases in

VO_{2max} following a combined training protocol after 12 and 16 weeks in HIV infected individuals (Robinson *et al*, 2007).

A significant reduction ($p < 0.05$) was observed in the body composition variables assessed in the exercise group. Since HIV-infected individuals receiving HAART are at risk for greater fat (subcutaneous and visceral) accumulation which may positively influence waist circumference, they represent a population at greater risk for metabolic abnormalities associated with cardiovascular disease (CVD) and diabetes. Therefore, significant reductions in waist circumference and percentage body fat in the study group may be a marker for decreased risk of metabolic diseases associated with abdominal obesity. This finding is in agreement with the report of the study by Tiozzo (2011) which showed a 2% decrease in waist circumference of HIV subjects treated with combined aerobic and resistance exercises. Also, Ogalha *et al* (2011) reported a significant decrease in BMI, body fat and muscle mass (increase) in a study to evaluate the impact of regular physical activity on patients with HIV. Filipas *et al* (2006) in a similar study of longer duration (6 months) reported a significant increase in muscle area and decreased waist circumference compared with the control group.

In addition to negative physical and physiological changes, HIV-infected patients receiving HAART can also experience psychological responses such as agitation, confusion, anxiety, nightmares, mania, and depression (Rosenfeld *et al*, 1999b). All the participants in this study showed significant increases in the QoL scores. These significant increases exist both within and between groups, but the change is more in the exercise group. The participants in the exercise group reported substantial improvements in both physical and mental quality of life scales while the participants in the control group had lower scores on the same scales. This indicates that the participants in the exercise group reported improvements in performing daily activities such as bathing, dressing, walking and climbing stairs which are activities captured in the physical quality of life scale.

Furthermore, higher mental quality of life scores observed in the exercise group indicate improved mental health with aerobic exercise and lower risk of depression (Stoll *et al*, 2001). This finding strongly agree with the findings of Ogalha *et al* (2011) in a similar study which showed a marked improvement in quality of life domains in both the exercise and non-exercise groups. However, this finding differs from the report of Tiozzo (2011) who observed improvement only in the exercise group and worsening quality life score in the control group. Ciccolo *et al* (2004) and Fillipas *et al* (2006) also found a significant improvement in the QoL domains of HIV participants using exercise intervention.

It is important to note that from the result of this study all participants received a clear benefit in terms of QoL evaluation, even those who were assigned to counseling sessions only. This fact suggests that the simple participation in an educational activity can promote a significant increase in QoL of patients with HIV/AIDS.

CONCLUSION

The results of this study showed that an eight-week aerobic exercise intervention coupled with nutritional guidance in people living with HIV/AIDS was able to significantly improve cardiovascular fitness, body composition and QoL. Nutritional guidance and counseling also brought about an improvement in cardiovascular fitness and QoL in people living with HIV/AIDS.

Clinical implications: Future trials should focus more upon longer duration exercise programmes for enhancing the general health status of individuals. It would be relevant to specifically target HIV patients of lower socio-economic status, since they represent individuals with greater susceptibility to disease progression and premature mortality (Cunningham *et al*, 2005). Studies with follow-up for longer period of time is required to define the duration of such effects and the need of a periodical "booster" to keep the benefits over time. It is important to include supervised exercise as a routine treatment in HIV/AIDS clinics. Physiotherapists should be involved in HIV/AIDS intervention programmes for supervised exercise programmes and management of other musculoskeletal impairments among this population.

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Table 1: **Socio-Demographic Characteristics of the Participants**

Variable	Control Group	Exercise Group
Age (yrs)	40.9 ± 8.0	41.0 ± 7.8
Height (m)	1.62 ± 0.74	1.6 ± .085
Gender		
Male	11 (40.1%)	8 (32%)
Female	16 (59.3%)	17 (68%)
Marital Status		
Single	10 (37%)	7 (28%)
Married	17 (63%)	18 (72%)
Educational level		
Secondary	16 (59.3%)	18 (72%)
Tertiary	11 (40.7%)	7 (28%)

Table 2: Comparison of Cardiovascular Variables between the Groups

Variables	Exercise group (n = 25) X±SD	Control group (n = 27) X±SD	t	p
Pre-study values				
SBP (mmHg)	123.0±15.78	125.0±15.67	-5.525	0.661
DBP (mmHg)	77.0±10.52	78.9±10.49	-3.280	0.701
RHR (bpm)	77.6±10.79	78.97±10.83	-2.423	0.164
$V0_2max$	41.4±5.73	40.5±4.17	0.643	0.525
Post-study values				
SBP (mmHg)	77.47±13.38	124.20±14.54	-7.888	0.001*
DBP (mmHg)	71.3±6.11	77.7±9.79	-7.055	0.001*
RHR (bpm)	72.4±6.63	78.03±10.01	-6.055	0.001*
$V0_2max$	65.0±9.05	40.60±4.24	12.75	0.001*

Key

SBP: Systolic Blood Pressure; **DBP:** Diastolic Blood Pressure; **RHR**: Resting Heart Rate,

$V0_2max$: Maximum Oxygen Consumption

*Significant

Table 3: Comparison of Cardiovascular Variables within Group

Variables	Pre-study X±SD	Post-study X±SD	t	p
Exercise Group				
SBP (mmHg)	123±15.79	117.0±13.36	6.378	0.001*
DBP (mmHg)	76.0±10.77	71.0±6.10	5.051	0.001*
RHR (bpm)	78.0±11.29	72.0±6.63	4.716	0.001*
Vo_2max	41.4±5.73	65.0±9.05	-20.376	0.001*
Control Group				
SBP (mmHg)	125.0±15.39	124.0±14.54	4.350	0.062
DBP (mmHg)	79.0±10.50	78.0±9.79	3.714	0.078
RHR (bpm)	79.0±10.83	78.0±10.0	3.080	0.124
Vo_2max	40.0±4.17	40.6±4.24	-0.279	0.782

Key
SBP: Systolic Blood Pressure; **DBP:** Diastolic Blood Pressure; **RHR**: Resting Heart Rate,
$V0_2max$: Maximum Oxygen Consumption
*Significant

Table 4: Comparison of Body Composition Variables between the Groups

Variables	Exercise Group (n = 25) X±SD	Control Group (n = 27) X±SD	t	p
Pre-study values				
BMI (kg/m^2)	27.39 ± 4.25	27.35 ± 5.10	0.039	0.969
BF (%)	30.52 ± 10.56	30.92 ± 9.92	-0.399	0.693
VF (%)	6.90 ± 1.61	7.93 ± 1.64	-2.079	0.047*
WC (cm)	82.53 ± 8.58	81.5 ± 8.58	0.444	0.661
Muscle (%)	30.45 ± 6.87	29.95 ± 6.59	0.280	0.781
Post-study values				
BMI (kg/m^2)	23.37 ± 3.55	27.2 ± 5.10	0.997	0.002*
BF (%)	27.0 ± 10.0	30.6 ± 10.10	-4.053	0.042*
VF (%)	6.0 ± 1.37	7.59 ± 1.68	-3.763	0.001*
WC (cm)	77.1 ± 6.59	81.27 ± 8.49	-3.663	0.002*
Muscle (%)	34.75±4.02	29.76±6.50	-3.456	0.001*

Key:
BMI: Body Mass Index; **BF**: Body Fat; **VF:** Visceral Fat; **WC**: Waist Circumference,
*Significant

Table 5: Within Group Comparison of Body Composition Variables

	Pre-study	**Post-study**	**t-test**	**p-value**
Exercise Group				
BMI (kg/m^2)	27.39 ± 4.25	23.37 ± 5.55	4.502	0.001*
WC (cm)	82.5 ± 8.58	77.1 ± 6.59	6.168	0.001*
VF (%)	6.9 ± 1.61	6 ± 1.37	-7.801	0.701
BF (%)	30.5 ± 6.87	27 ± 10	6.400	0.001*
Muscle (%)	30.4 ± 6.87	34.75 ± 4.02	-6.196	0.001*
Control Group				
BMI (kg/m^2)	27.3 ± 5.08	27.2 ± 5.10	0.096	0.960
WC (cm)	81.5 ± 8.53	81.27 ± 8.49	1.523	0.961
VF (%)	7.93 ± 1.64	7.59 ± 1.68	0.208	0.680
BF (%)	30.92 ± 9.92	30.6 ± 10	1.494	0.680
Muscle (%)	29.95 ± 6.59	29.76 ± 6.50	0.417	0.680

Key:
BMI: Body Mass Index; **BF**: Body Fat; **VF:** Visceral Fat; **WC**: Waist Circumference,
*Significant

Table 6: Between Group Comparisons of QoL Scores

	Exercise group (n = 25) X±SD	**Control group (n = 27) X±SD**	**t – value**	**p - value**
Pre-study values				
General Health	26.17 ± 5.68	21.17 ± 6.11	-0.612	0.545
Physical Functioning	38.97 ± 4.97	40.1 ± 5.33	-2.152	0.060
Role functioning	20 ± 24.90	25 ± 25.43	-0.828	0.415
Cognitive Functioning	42.7 ±17.90	34.83 ± 9.10	1.932	0.063
Mental health	23.77 ± 11.33	27.33 ± 11.43	-1.426	0.164
Energy/vitality	26.67 ± 5.46	29.5 ± 8.13	-1.598	0.121
Post-study values				
General health	51.33 ±3.93	35 ± 5.41	13.927	0.001*
Physical Functioning	92.47 ±6.45	67.1 ± 9	12.868	0.001*
Role functioning	73.33 ± 31.44	41.67 ±32.37	3.898	0.001*
Cognitive Functioning	100 ±00	72.67 ± 8.17	18.320	0.001*
Mental health	71.37 ± 3.71	60.37 ± 6.67	9.090	0.001*
Energy/vitality	59.67 ± 4.72	47 ± 7.38	8.382	0.001*

*Significant at $p<0.05$

Table 7: Within group comparison of QoL scores

	Pre-study	**Post-study**	**t – value**	**p – value**
Exercise group				
General health	26.2 ± 5.68	51.3 ± 3.93	-20.796	0.001*
Physical Functioning	37.3 ± 4.98	92.5 ± 6.45	-55.559	0.001*
Role functioning	20 ± 24.91	73 ± 31.44	-7.899	0.001*
Cognitive functioning	42.7 ± 17.90	100 ± 00	-17.523	0.001*
Mental health	23.8 ± 11.30	71.4 ± 3.71	-21.682	0.001*
Energy/vitality	26.7 ± 5.47	59.7 ± 4.72	-26.690	0.001*
Control group				
General health	27.2 ± 6.10	35 ± 5.41	-6.861	0.001*
Physical Functioning	40 ±5.33	67 ± 9.03	-14.628	0.001*
Role functioning	25 ± 25.43	41.7 ± 32.39	-3.340	0.001*
Cognitive Functioning	34.8 ± 9.05	72.67 ± 8.17	-19.776	0.001*
Mental health	27 ± 11.43	60.4 ± 6.67	-16.938	0.001*
Energy/vitality	29.5 ± 8.10	47 ± 7.38	8.5558	0.001*

* Significant

RELATIONSHIP BETWEEN QUALITY OF LIFE AND PARTICIPATION RESTRICTION OR ACTIVITY LIMITATION AMONG CHILDREN WITH SICKLE CELL DISEASE

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ABSTRACT
Background: Sickle cell disease (SCD) brings about health complications that affect capacity to participate in physical activities of daily living. Little is known about the relationship between quality of life and participation restriction as well as activity limitation among children with SCD, hence the reason for this study. **Methodology:** One hundred and seventy five children with SCD selected by purposive sampling technique from selected hospitals with SCD Centers in Lagos state, participated in this cross sectional descriptive study. Their care givers completed two paediatric questionnaires namely 'Paediatric quality of life, disability and co morbidity inventory child report (23 items) and sickle cell disease quality of life questionnaire (27 items)' on their behalf. Data was analysed using SPSS version 17 and summarized using descriptive analysis of mean, percentage and frequency. Spearman rho's correlation coefficient was used to compare variables and results were presented in tables. The level of significance was set at $p < 0.05$. **Results:** The mean age of subjects was 9.06 + 1.63 years. The quality of life of most of the children with SCD was observed to be reduced. A positive correlation was obtained between the quality of life and participation restriction as well as activity limitation of these children with SCD. These correlations were also statistically significant ($r = 0.39$, $p = 0.001$; $r = 0.21$, $p = 0.01$). **Conclusion:** The reduced quality of life among children with SCD may be due to participation restriction and activity limitation in normal life activities.

Keywords: *Sickle cell disease, Quality of life, Participation restriction, Activity limitation.*

INTRODUCTION

Sickle cell disease (SCD) is a common genetic condition due to an inheritance of mutant haemoglobin genes from both parents. The mutation in the parental gene coding for haemoglobin (HS) has valine substituted for glutamic acid in the sixth amino acid of the beta globin chain.[1] The greatest burden of SCD is in sub-Saharan Africa (SSA), where 75% of the 300,000 global births of affected children live.[2] This is because one-third of the indigenous inhabitants of SSA carry the gene.[3] Other places where it can be found include the Middle East, Greece, Turkey, the Mediterranean, North and South America.[4] It is incurable and about 50% to 80% of the children with the disease will die before adulthood.[5]

The clinical manifestations are diverse and may include vaso-occlusive, hematological and infectious crises.[6] Children with SCD manifest the features of chronic anemia, including pale skin and mucous membranes, fatigue, frequent infections, poor wound healing and often have a yellowing of their eyes and skin (jaundice).[7] There are usually periodic episodes of severe pain in the joints, muscles, chest, or abdomen (sickle cell crisis).[8]

Treatment advances in recent decades have now transformed SCD into a chronic disease suffered by children and adults.[9] Frequently, patients surviving until adulthood experience significant organ system damage that may include stroke, pulmonary failure and pulmonary hypertension, renal failure, congestive heart failure, leg ulcers, and osteonecrosis of the femoral or humeral

heads.[10] These health complications such as stroke may impair function which may reduce the capacity of these children to participate in physical activities and may even precede death.[11]

Health-related quality of life (HRQOL) is deteriorated by episodic, debilitating pain associated with substantial analgesic use, frequent hospitalization for pain episodes, and ultimately organ failure.[12,13] Children with this disease are far worse, in their HRQOL when compared to assessment of general physical, motor and independent daily functioning.[14] Little is known about the relationship between quality of life and participation restriction as well as activity limitation among children with SCD, hence the reason for this study.

METHODS

One hundred and seventy five children (89 males and 86 females) with SCD whose ages ranged from 7 years to 12 years participated in this cross sectional descriptive study. They were selected by purposive sampling technique from the Medicine Outpatient and Paediatric clinics of the Lagos University Teaching Hospital (LUTH), Idi-Araba, Lagos, Lagos state University Teaching Hospital (LASUTH) Ikeja, Lagos, General Hospital, Gbagada, Lagos, Massey Children Hospital and Sickle Cell Area Clubs of Lagos State located at Yaba, Isolo, Lagos Island, Apapa, Festac, Surulere and Ikorodu.

Ethical approval was obtained from the Health Research and Ethics Committee of the Lagos University Teaching Hospital, Idi-Araba, Lagos. An informed consent was obtained from the parent/guardian of each of the subjects who also completed the paediatric questionnaires on their behalf.

Questionnaire Design

Two validated paediatric questionnaires namely 'Paediatric quality of life, disability and co morbidity inventory child report and sickle cell disease quality of life questionnaire adapted from previous study by Crystal[15] were used for this study. Paediatric quality of life, disability and co morbidity inventory child report has 23 items presented in five domains. First domain (3 items) has questions on participation restriction of the child's activities of daily living. Second domain (5 items) has questions on how SCD causes activity limitation for the child on a daily basis. Third domain (5 items) has questions on how SCD affects the child's emotions. Fourth domain (5 items) has questions on how SCD affects the child's relationship with other children. The Fifth domain (5 items) deal with the child's thinking ability in school. These domains are based on 5-point scale (0= Never a problem to 4= Almost always a problem). The sickle cell disease quality of life questionnaire has questions on how SCD affects the child's quality of life and consisted of 27 items whose scores ranged from (1= Always to 4= Never).

Administration of Questionnaire

The aims and objectives of the study were clearly explained to the parents/guardians of the subjects by the researchers and were also assured of the confidentiality of their responses. Those who gave their consent were given the questionnaires to fill. The collection of the completed questionnaires was done immediately by hand by.

Data analysis

Data was analysed using SPSS version 17 and summarized using descriptive analysis of mean, standard devation, percentage and frequency. Spearman rho's correlation coefficient was used to compare variables and results were presented in tables. The level of significance was set at $p < 0.05$.

RESULTS

A total of 175 copies of the questionnaire were distributed and all the copies were returned giving a response rate of 100%. Thirty eight (21.7%) of the subjects were aged 7 years and the mean age of subjects was 9.06 + 1.63 years (Table 1). One hundred and eight (61.7%) respondents scored between 25 and 44 in the quality of life scores (Table 2). The higher the score the higher the quality of life while the lower the score the lower the quality of life.

One hundred and six (60.6%) scored between 0 and 5 in the participation restriction distribution domain (Table 3). The higher the score the more they are restricted from participating in normal activities (eg sports, running). Eighty four (48.0%) scored between 6 and 10 in the activity limitation domain (Table 4). The higher the score, the more limited they are in carrying out normal activities.

One hundred and fourteen (65.1%) respondents scored between 0 and 5 in the emotional domain (Table 5). The higher the emotional score the higher their emotional problems. One hundred and twenty-six (72.0%) respondents scored between 0 and 5 in the relationship domain (Table 6). The higher the relationship domain score, the lower the relationship with their peers. Eighty one (46.3%) respondents scored between 0 and 5 in the intellectual domain (Table 7). The higher the intellectual score, the higher the respondents' problem intellectually.

Spearman rank order correlation test showed a significant relationship between age and quality of life (p=0.03). The respondents between the age ranges of 7 – 8 years experienced lower quality of life (Table 8). Spearman rank order correlation test showed no significant relationship between sex and quality of life (p=0.72). Spearman rank order correlation test showed a significant relationship between quality of life and participation restriction (p=0.001). The respondents that were more restricted from participating in activities experienced lower quality of life. Spearman rank order correlation test showed a significant relationship between quality of life and activity limitation (p=0.01). The respondents who were more limited in performing activities of daily living experienced lower quality of life (Table 8).

Spearman rank order correlation test also showed a significant relationship between quality of life and emotional state; relationship with peers and intellectual levels of children with SCD (p=0.001) (Table 9).

DISCUSSION

This study was designed to determine the relationship between quality of life, participation restriction and activity limitation among children with sickle cell disease. The quality of life of most of the children with SCD was observed to be low (33.1% of participants) and moderate (61.7% of participants). This implies that the quality of life of children with SCD in Lagos state, Nigeria was reduced below normal. Crystal[15] reported that the quality of life of 78.7% of African American children with SCD was reduced.

The observation that 60.6% of children with SCD had a very low score of 0-5 in the participation restriction domain implies that the participation of these children in physical activities of daily living and sports is very restricted. Again, the observation that 30.9% of children with SCD had a very low score of 0-5 and 48% had a score of 6-10 in the activity limitation domain implies that these children are limited in carrying out activities of daily living. These may be due to the various limitations and health challenges imposed upon them by the disease. Brambilla *et al*[11] stated that health complications such as stroke in children with SCD may impair function and may reduce their capacity to participate in physical activities and may even precede death. Lewandowski[16] stated that people with SCD who had headaches, arthritis etc, reported significantly greater limitation at school attendance, reading, school work and playing with friends and in sports.

It was observed that there was a significant relationship participation restriction and quality of life. This implies that the higher the participation restriction, the lower the quality of life. Fuggie *et al*[17] reported that the decreased participation in physical activities and exercises by children with SCD reduced their quality of life when compared to the control children they studied. Maikler[18] stated that children with SCD had decreased participation in all activities (school, sports, play, social) and this depressed them and decreased their quality of life.

It was also observed that there was a significant relationship between quality of life and activity limitation. This implies that the higher the activity limitation, the lower the quality of life. Lewandoswski[16] reported a significant relationship between quality of life among children with SCD and activity limitation. For example acute pain episodes are associated with significant physical symptoms that have been found to interfere with school attendance and interactions with peers which may lead to decreased quality of life in the physical, social, and/or academic domains.[19]

It was observed that there was a significant relationship between age and quality of life of children with SCD. This implies that the age of children with SCD affected their quality of life. Annett[20] reported that there was a significant relationship between age and quality of life. She advised that when examining quality of life in children, it is also important for researchers to recognize that development may play an important role. She further postulated that it is possible that a similar relationship might exist between age and perceptions of quality of life. Specifically, it is thought that due to their stable and self-focused cognitions younger children may not realize that their health may change over time, which may lead them to misperceive the cause of their illness.[21] Thus, these cognitions and misperceptions may impact their report of their own quality of life. For example, due to egocentricism younger children may think that their physical limitations are due to something they did wrong.[21]

It was also observed that there was no significant relationship between gender and quality of life of children with SCD. This implies that the sex of children with SCD had no effect on their quality of life. Crystal[15] reported that there were significant differences in quality of life between males and females with SCD. Specifically, males reported higher overall generic and disease-specific quality of life compared to females.

It was observed that there was a significant relationship between quality of life and the emotional state of children with SCD. This implies that as the lower the emotional state of children with SCD, the lower their quality of life. It was also observed that there was a significant relationship between quality of life and SCD children's relationship with their peers. This implies that the lower the relationship with their peers, the lower their quality of life. It was observed that there was a significant relationship between quality of life and intellectual level of children with SCD. This implies that the lower their intellectual level, the more they feel inferior to their peers who do not have SCD which leads to depression and hence a lower quality of life.

CONCLUSION

The reduced quality of life among children with SCD may be due to participation restriction and activity limitation in normal life activities. The reduced emotional state, relationship with peers and intellectual level may have also contributed to the reduced quality of life among these children.

Relevance of the Study

Awareness campaign on the importance of physical activity for children with SCD should be disseminated through seminars by physiotherapists to parents, caregivers and teachers. They should be advised not to unduly restrict these children with SCD from participating in normal activities but they should be allowed to take part in physical activities of interest to them with close monitoring.

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Table 1: Ages of respondents

Age (years)	Number of respondents (N)	Percentage (%)	Male (N)	Male (%)	Female (N)	Female (%)	Mean + SD
7	38	21.7	20	22.5	18	20.9	
8	37	21.1	17	19.1	20	23.3	
9	33	18.9	20	22.5	13	15.1	
10	28	16.0	17	19.1	11	12.8	
11	20	11.4	8	9.0	12	14.0	
12	19	10.9	7	7.8	12	14.0	
							9.06 1.63
Total	**175**	**100**	**89**	**100**	**86**	**100**	

Table 2: Sickle cell Quality of life score of the respondents

Score	Number of respondents (N)	Percentage (%)
7-24	58	33.1
25-44	108	61.7
45-64	9	5.2
Total	**175**	**100**

Table 3: Participation restriction scores of the respondents

Score	Number of respondents (N)	Percentage (%)	Male (N)	Male (%)	Female (N)	Female (%)
0-5	106	60.6	58	65.2	46	55.8
6-10	62	35.4	29	32.6	33	38.4
11-15	7	4.0	2	2.2	5	5.8
Total	**175**	**100**	**89**	**100**	**86**	**100**

Table 4: Activity limitation scores of the respondents

Score	Number of respondents (N)	Percentage (%)	Male (N)	Male (%)	Female (N)	Female (%)
0-5	54	30.9	28	31.5	26	30.2
6-10	84	48.0	43	48.3	41	47.7
11-15	25	14.3	15	16.9	10	11.6
16-20	12	6.9	3	3.4	9	10.5

Total	**175**	**100**	**89**	**100**	**86**	**100**

Table 5: Emotional scores of the respondents

Score	Number of respondents (N)	Percentage (%)	Male (N)	Male (%)	Female (N)	Female (%)
0-5	114	65.1	59	66.3	55	70.0
6-10	45	25.7	22	24.7	23	26.7
11-15	25	14.3	15	6.9	10	11.6
16-20	12	6.9	3	3.4	9	10.5
Total	**175**	**100**	**89**	**100**	**86**	**100**

Table 6: Relationship (how the children get along with others) score of the respondents

Score	Number of respondents (N)	Percentage (%)	Male (N)	Male (%)	Female (N)	Female (%)
0-5	126	72.0	62	69.7	64	74.4
6-10	40	22.8	23	25.8	17	19.8
11-15	8	4.6	3	3.4	5	5.8
16-20	1	0.6	1	1.1	0	0.0
Total	**175**	**100**	**89**	**100**	**86**	**100**

Table 7: Intellectual score of the respondents

Score	Number of respondents (N)	Percentage (%)
0-5	81	46.3
6-10	77	44.0
11-15	11	6.3
16-20	6	3.4
Total	**175**	**100**

Table 8: Spearman correlation relationship between age and quality of life, gender, participation restriction and activity limitation

	7-24		Quality of Life scores				Total		rs	p-value
	N	%	25-44 N	%	45-64 N	%	N	%		
Age (Yrs)										
7-8	29	38.7	42	56.0	4	5.3	**75**	**100**		
8-9	20	32.8	40	65.6	0	0.0	**61**	**100**		
10-12	9	23.1	26	66.7	5	12.8	**39**	**100**		
									0.7	0.03
Total	**58**		**108**		**9**		**175**			
Gender										
Male	26	29.2	58	65.2	5	5.6	**89**	**100**		
Female	32	37.2	50	58.1	4	4.7	**86**	**100**		
									0.27	0.72
Total	**58**		**108**		**9**		**175**	**100**		
Participation restriction										
0-5	45	42.5	61	57.5	0	0.0	**106**	**100**		
6-10	13	21.0	42	67.7	7	11.3	**62**	**100**		
11-15	0	0.0	5	71.4	2	28.6	**7**	**100**		
									0.39	0.001
Total	**58**		**108**		**9**		**175**	**100**		
Activity limitation										
0-5	23	42.6	31	57.4	0	0.0	**54**	**100**		
6-10	22	26.2	60	71.4	2	2.4	**84**	**100**		
11-15	11	4.4	10	4.0	4	16.0	**25**	**100**		
16-20	2	11.7	7	58.3	32	5.0	**12**	**100**		
									0.21	0.01
Total	**58**		**108**		**9**		**175**	**100**		

Significance level set at $p < 0.05$

Table 9: Spearman rho correlation of the domain variables

Variables	Rs	p-value
Relationship	0.37	0.001
Emotion	0.50	0.001
Intellectual	0.39	0.001

Significance level set at $p < 0.05$

VERIFICATION OF A TREATMENT PLANNING SYSTEM USING AN IN-HOUSE DESIGNED TRUNK PHANTOM

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ABSTRACT

Background and Purpose: A phantom for use in radiotherapy treatment planning of human trunk anatomical region has been designed with six hollows for inserting materials mimicking different biological tissues and the ionization chamber. The purpose of the study is to verify the accuracy of the Irregular Field Algorithm by using an Elekta-Precise Clinical Linear Accelerator of 6MeV Photon beam with small and large field sizes. **Material and Methods:** The study was executed with a designed in-house phantom made of Plexiglas. The phantom was scanned using a Hi-Speed CT scanner and TPS for image verification. 100% Pure Glycerol was used for Muscle, 75% : 25% Glycerol to Water was used for liver, 100% of Carboxyl-Methyl-Cellulose (CMC) was used for Lungs, 50% : 50% Glycerol to Water was used for Adipose, 100% of Sodium Hypochlorite (soda bleach) was used for Bone and 100% of Sodium Laureth Sulphate (Texapon) was used for Kidney. **Results:** The maximum percentage deviation with large field sizes for six inhomogeneous inserts and with bone only inhomogeneous inserts were -3.39% and 2.93% respectively. The maximum percentage deviation with small field size with six inhomogeneous inserts was -3.16%. The percentage deviation between the solid water phantom and the in-house phantom was -3.35%. **Conclusion:** In summary, the Irregular Field Algorithm has shown an overall accuracy of $\pm 3\%$ with the in-house phantom for small and large field sizes. These values are well within clinical tolerance levels.

Keywords: *Phantom, Treatment Planning System (TPS), Irregular Field Algorithm, Clarkson Integration, Computed Tomography.*

INTRODUCTION

Radiotherapy aims to cure, or locally control disease, while concurrently minimizing complications in normal tissue. The International Commission on Radiation Units and Measurements (ICRU) has recommended that radiation dose must be delivered to within $\pm 5\%$ of the prescribed dose [2, 10, 15]. For a centre using a conventional treatment technique, which is based primarily on measured data [14], there is the need to verify the algorithm in use because quality assurance program ensures that all the components of the treatment facilities used in radiotherapy must be properly checked for accuracy and consistency and that all radiation generating facilities are functioning according to manufacturer's specification. Several technique of carrying out the quality assurance of TPS has been proposed by various authors [7, 8, 16, 17, 18, 19]. Likewise, the reduction of errors and uncertainties in the dose calculation plays an important role in the success of a treatment procedure [6, 9, 11, 18].The performance and quality of any Treatment Planning System (TPS) is dependent on the type of algorithm used. An algorithm is defined as sequence of instructions that operate on a set of input data, transforming that information into a set of output result that are of interest to the user [3] .Treatment planning requires the ability to calculate dose to any arbitrary point, within the patient, for any given beam orientation. In this study, the Irregular Field Algorithm was used. Irregular Field Algorithm requires the separation of the dose into primary and scatter components. The concept of this

dosimetry of irregular fields using TMRs and SMRs is analogous to the method using TARs and SARs [14].The magnitude of the dose from scattered radiation at some given point can be quantified using the Scatter-Air or Scatter-maximum Ratios (SARs, SMRs). Equation 1 explains this Irregular Field Algorithm which is based on Clarkson Integration. The program equation is given by:

$$Dose\ Rate = TRAY \cdot TRAY2 \cdot OUTPUT \cdot FSC \cdot (P \cdot OCR \cdot QF \cdot TAR0 + SC) \cdot \frac{(SSD+DMAX+c)^2}{X^2+Y^2+(SPD+c)^2} \quad (1)$$

Where:
TRAY and TRAY2 = are the tray factors.
OUTPUT = the output factor for 0×0 field size
FSC = the air field size correction dependency factor, computed for equivalent square of the collimator opening
SSD = source to surface distance
DMAX = Dose at maximum
SPD = source to point distance of calculation
X and Y = co-ordinates at depth of the point of calculation
c = is the correction for virtual location of the source
QF = quality factor
OCR = off- centre ratio
TAR0 = tissue-air ratio at the surface

METHODS

The designed in-house phantom was made of Plexiglas of thickness 0.33mm having a density 1.16 g/cm^3 [14]. A plastic based hardener (allplast) was used for holding one slab to another to form a cube. The Plexiglas used was purchased from a local plastic shop of dimension 4 by 8 feet, a part of which was cut using a plastic cutter into six slabs each of dimension 30×30 cm . Seven holes were drilled on one face. Each drilled hole had a diameter of 2.5cm gummed together using plastic based hardener called ‘allplast’. Before the holes were drilled, the distance from the surface of the designed in-house phantom to the ionization chamber was 15cm, while the distance between two diagonal insert were approximately 22cm. The distances from one insert to the other (horizontally) was 7cm and vertically were 18cm. Also additional drilled hole was made at the top of the designed in-phantom to allow for easy filling of water and evacuation of water from the phantom. After these holes and distance have been marked out, another cylindrical rod made of Plexiglas material of thickness 0.2mm, length 14.3cm and diameter 2.5cm were fitted into the seven drilled holes and were held together at the tip by the ‘allplast’ gum to avoid leakage. A view of this designed in-house phantom is shown in figure (A). Also, the in-house phantom was loaded with tissue-equivalent material putting into consideration the attenuation coefficients, electron densities and the effective atomic numbers of each chemical composition as shown in table (A). The in-house phantom was scanned under a Hi-Speed CT-scanner. Slices of images were acquired for six different tissue-equivalent materials as shown in figure (B). A second scan was conducted for bone only as shown in figure (C). From the acquired CT images, inhomogeneities were determined using Computed Tomography number calculation algorithm. The scanned images were transferred to the precise PLAN Treatment Planning System for beam application as shown in figure (D), (E) and (F). Large field size ($22\times24cm^2$) and small field size ($5\times5cm^2$) were used for this study, with the gantry head moving between range [0°, 22.5° , 45° , 90° , 135°, 157.5°, 180°, 197.5° , 215° , 270°, 315° , 337.5°] for the twelve fields and [0° , 45° , 90° , 180° , 225° , 270°]for the six fields. The plans in figure (D), (E) and (F) were later transferred from the Elekta-precise PLAN Treatment

Planning System to the Elekta-Precise Clinical Linear Accelerator through a DICOM for treatment.

Furthermore, a simple experimental protocol for the verification of the algorithm was performed between the in-house phantom and the solid water phantom with Source to Surface Distance (SSD) of 85cm depth. Six readings were observed with the gantry head at 0°. According to this study, the Irregular Field Algorithm which is based on previously published methods [5, 12, 13] was configured to give 1.0 Gy at the iso-centre. The plans were then used with the pre-calibrated Elekta-Precise clinical linear accelerator for measurements. Measurements were conducted using 6 MeV photon beams from the Elekta-Precise Clinical Linear Accelerator with iso-centric set up. A pre-calibrated NE 2570/1 farmer-type ionization chamber along with its electrometer was used to determine the absorbed dose in Gray. Necessary corrections for temperature, pressure, polarization, recombination etc were effected on the ionization chamber response. Five measurements were made in all, four for the in-house phantom and one for the solid water phantom. Absorbed dose at reference depth was calculated as follows [1]:

$$D_{W,Q} = M_Q \times N_{D,W} \times K_{QQ_o} \quad (1)$$

Where M_Q is the electrometer reading (charge) corrected for temperature and pressure, $N_{D,W}$ is the chamber calibration factor and K_{Q,Q_o} is the factor which corrects for difference in the response of the dosimeter at the calibration quality Q and at quality Q_0 of the clinical x-ray beam according to the TRS 398 protocol of the IAEA.
Deviation between the calculated and measured dose was obtained using the equation as:

$$\% \text{ Deviation} = \frac{D_c - D_m}{D_m} \times 100 \quad (2)$$

Where:

D_c = Calculated dose

D_m = Measured dose

RESULTS

Table (B) describes the outcome of the treated in-house phantom with large field size with twelve field plan for six inhomogeneous inserts where the absorbed dose reading (ADR) were averaged and the corresponding standard deviation and percentage deviations were calculated. Table (C), also describes the outcome of the treated in-house phantom with large field size with six field plan for bone only, six inhomogeneous inserts where the absorbed dose reading (ADR) were averaged and the corresponding standard deviation and percentage deviations were calculated. Table (D), describes the outcome of the treated in-house phantom with small field size with six field plan for six inhomogeneous inserts where the absorbed dose reading (ADR) were averaged and the corresponding standard deviation and percentage deviations were calculated. Table (E), compares the absorbed dose reading for the solid water phantom of the Elekta-Precise Clinical Linear Accelerator against that of the in-house phantom.

DISCUSSION

A study has been carried out to verify the performance of a Treatment Planning System which uses an Irregular Field Algorithm based on previously published methods [5, 12, 13]. The results

were within the range of $\pm 5\%$ as recommended by ICRU [10] and were consistent with Van Dyk whose variation was within $\pm 4\%$, [18]. Mijnheer and Brahme were within $3-3.5\%$ [4, 15]. The results in table (B) and table (C) showed that the Irregular Field Algorithm had a maximum percentage deviation of -3.39% and $+2.93\%$ for six inhomogeneous inserts and bone only inhomogeneous inserts with large field size of $22 \times 24 cm^2$ respectively. The result for table (D) for six fields with six inhomogeneous inserts with small field size of $5 \times 5 cm^2$ had a maximum percentage deviation of -3.16%. The result for table (E) showed that the percentage deviation between the solid water phantom and the in-house phantom was -3.33%, which is approximately equal to the result obtained in table (B) and (C) respectively, this further confirms the accuracy of the in-house phantom. Table (B) showed an increase in numerical value for the first five fields, with the highest deviation noticed in the tenth field. The overall percentage deviation range was $0.04\% \text{ to } -3.39\%$. In table (B), the least percentage deviation was noticed in the sixth field with a value of -0.85%, with an overall percentage deviation range of $-0.85\% \text{ to } +2.93\%$. In addition, the least deviation were observed for the first field in table (B) and second field in table (D) whose value was ±0.04%.An overview of this study showed that the designed in-house phantom was within approximately ±3%. These values are well within clinical tolerance levels, making this in-house phantom fit for use in radiotherapy department at large.

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Table 1. Chemical Composition

Tissue Equivalent Material	Elemental Composition
Liver	75% of Glycerol : 25% of Water
Lungs	100% Carboxyl Methyl Cellulose (CMC)
Muscle	100% of Glycerol
Adipose	50% of Glycerol : 50% of Water
Bone	100% Sodium Hypochlorite (soda bleach)
Kidney	100% of Sodium Laureth Sulfate

Table 2. Measured Absorbed dose (Gy) and Percentage Deviation for twelve fields with six Inhomogeneous Inserts with Field Size of 22×24cm^2

FIELD	ADR 1	ADR 2	ADR 3	STD	AVG	%DEV
1	0.9996	0.9996	1.0019	0.0013	1.0004	0.04
2	0.9973	0.9973	0.9996	0.0013	0.9981	-0.19
3	0.9969	0.9954	0.9999	0.0023	0.9974	-0.26
4	1.0019	1.0041	1.0042	0.0013	1.0034	0.34
5	0.9734	0.9813	0.9734	0.0046	0.9760	-2.40
6	1.0096	1.0055	1.0096	0.0024	1.0082	0.82
7	0.9758	0.9563	0.9758	0.0113	0.9693	-3.07
8	0.9722	0.9723	0.9722	0.0001	0.9722	-2.78
9	0.9766	0.9835	0.9858	0.0048	0.9820	-1.80
10	0.9676	0.9745	0.9562	0.0092	0.9661	-3.39
11	0.9769	0.9769	0.9586	0.0106	0.9708	-2.92
12	0.9836	0.9815	0.9746	0.0047	0.9799	-2.01
ADR= Absorbed Dose Reading						

Table 3. Measured Absorbed dose (Gy) and Percentage Deviation for six fields with six bone only Inhomogeneous Inserts with Field Size of 22×24cm^2

FIELD	ADR 1	ADR 2	ADR 3	STD	AVG	%DEV
1	1.0300	1.0300	1.0280	0.0012	1.0293	2.93
2	1.0166	1.0166	1.0189	0.0013	1.0174	1.74
3	1.0234	1.0211	1.0257	0.0023	1.0234	2.34
4	1.0120	1.0166	1.0052	0.0057	1.0113	1.13
5	1.0119	1.0142	1.012	0.0013	1.0127	1.27
6	0.9915	0.9915	0.9915	0.0000	0.9915	-0.85
ADR= Absorbed Dose Reading						

Table 4. Measured Absorbed dose(Gy) and Percentage Deviation for six fields with six Inhomogeneous Inserts with Field Size of 5×5cm^2

FIELD	ADR 1	ADR 2	ADR 3	STD	AVG	%DEV
1	1.0064	1.0041	1.0064	0.0013	1.0056	0.56
2	0.9996	0.9996	0.9996	0.0000	0.9996	-0.04
3	0.9859	0.9836	0.9836	0.0013	0.9844	-1.56
4	0.9677	0.9699	0.9677	0.0013	0.9684	-3.16
5	0.9760	0.9760	0.9650	0.0064	0.9723	-2.77
6	0.9765	0.9734	0.9765	0.0018	0.9755	-2.45
ADR= Absorbed Dose Reading						

Table 5. Comparism between solid water phantom and designed in-house trunk phantom

	Solid water phantom (Gy)	**Designed phantom (Gy)**
	0.6282	0.6076
	0.6282	0.6076
	0.6260	0.6054
	0.6282	0.6054
	0.6260	0.6054
	0.6260	0.6076
AVG	0.6275	0.6065
STD	0.0011	0.0012
	%DEV= -3.35	

ASSESSMENT OF ULTRASOUND EQUIPMENT AS A POSSIBLE SOURCE OF NOSOCOMIAL INFECTION IN LAGOS STATE HOSPITALS AND RADIO-DIAGNOSTIC CENTRES

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ABSTRACT
Aim: To assess the role of ultrasound equipment as a possible source of nosocomial infections in Lagos State Hospitals and diagnostic centres and to identify the types of Microorganisms found in the equipment. **Methods:** Microbiological cultures were carried out on samples obtained from ultrasound probes, gel and couch before and after scanning period. Cultures were incubated in a culture plate (Chocolate and MacConkey agar) for 48 hours at a temperature of 370 in order to grow microorganism, after which the culture plate was examined microscopically against a bright light in order to identify the isolated organisms based on their colonial characteristics. **Results:** Transabdominal ultrasound probes, transvaginal probe, ultrasound couch and ultrasound gel all were contaminated with microorganisms. Staphylococcus aureus was the most frequent and most common organisms found (33.8%). Other organisms such as Staphylococcus epidermidis (15.4%), Candida albicans (6.2%), aerobic spore formers (26.2%), Klebsiella pneumonia (6.2%), Pseudomonasaeruginosa (3.1%), among others were also identified. **Conclusion:** Ultrasound equipment is a possible source of nosocomial infection for patient undergoing ultrasonography. The most commonly isolated bacterial were staphylococcus aureus, Staphylococusepidermidis, Aerobic spore formers, among others according to this research.

Keywords: *Ultrasound, Nosocomial infection, Contamination, ultrasound transducer, coupling gel*

INTRODUCTION

Nosocomial infections are terms often used interchangeably with hospital acquired infection. A nosocomial infection is an infection a patient contracts during hospitalization which was neither present nor incubating at the time of his/her admission. It is also referred to as an infection that first appears between 48 and 72 hours after a patient is admitted to a hospital or a health care facility [1]. Nosocomial infections have long been recognized as a major public health problem globally [2] and are reported to be rising especially in resource-poor countries of the sub-Sahara Africa, and are important contributors to morbidity and mortality [3]. It is believed that they will become even more important as a public health problem with increasing economic and human impact because of increasing population, crowding of people and as the number of people with impaired immunity due to their age, illness and treatments increase. Furthermore, it has been suggested that the emergence of new microorganisms and increasing bacterial resistance to antibiotics will make nosocomial infections even more serious public health problems [4]. And quite unfortunately, little progress has been made, according to the World Health Organization (WHO), in addressing the basic problems responsible for the rapid increase in the incidence of nosocomial infections during the past 10-20 years. In 2010, for instance, the World Health Organization (WHO) reported that >50% of patients become infected during hospitalization [5]. Nosocomial infections are reported to be more prevalent in resource-poor countries in Sub-Sahara Africa where preventable disease such as diarrhea, sexually infections and HIV/AIDS are

endemic [6]. Moreover, it has been suggested that at least 80% of patients in developing countries acquires nosocomial infections and reasons given for the reported rise in the incidence of nosocomial infection include drug resistance, lifestyle changes and increased use of invasive procedures [4]. In this era of evidence-based medical practice, many an ultrasound center is a beehive of activity daily as more patients are currently being referred for different ultrasound investigations by clinicians who require accurate diagnosis as basis for treatment of diseases. The ultrasound section of the radiology department therefore, understandably plays host to a large number of patients some of who come to the ultrasound center with open wounds, draining sores and drainage tubes as well as life support machines. And more often than not, the ultrasound gel and probe must make contact with patient's skin during sonographic examination. It has been reported that transmission of most infections can be prevented with readily available, relatively inexpensive aseptic strategies such as cleaning of ultrasound probe and couch with alcohol-based sanitizers [7]. This appears not to be the case in the metropolitan Lagos as most practitioners only wipe their probe with either a dry paper towel or napkin after use. It is also not uncommon to observe some sonographers who do not even ensure that the probe is thoroughly wiped clean before another patient is brought in. Such 'unsafe use of medical equipment' could make the ultrasound equipment, probe and gel likely veritable sources of nosocomial infection [7]. In fact, a note of warning had been sounded that 'with increasing use of ultrasound in medical diagnosis, there is the potential for transmission of nosocomial infection via the ultrasound probe, ultrasound couch and also the coupling gel'[8]. In several studies, different medical equipment and accessories have been implicated as habouring pathogens [9-12] and as such, may be involved in transmission of infections. It is therefore necessary to ascertain the role of of ultrasound equipment as a possible source of nosocomial infection in Lagos State Nigeria.

METHODS

In the longitudinal study, convenience sampling method was used to select 3 ultrasound centers in Lagos metropolis. These are the ultrasound centers of Lagos University teaching Hospital (LUTH), Idi-araba, Life Channel Diagnostic Centre (LCDC), Fagba-Iju and Esteem Medical Diagnostic Services Limited (EMDSL), Ebute-Meta Lagos.

Data Collection

A pathologist and a laboratory scientist with 15 and 10 years' experience respectively were recruited to help in data collection and in the reading of culture. A total of 36 swab samples were aseptically collected using sterile swab sticks from ultrasound probes, couches and coupling gel in the 3 ultrasound centers. Swabs were collected aseptically from the surface of ultrasound probes and couches before and after scanning periods. Swabs were taken immediately after scanning a patient and in the middle of the scanning period. The swabs were appropriately labeled and were taken to the laboratory immediately for culture on MacConkey and chocolate agar for isolation of pathogens.

Preparation of cultural media

All culture (MacConkey and chocolate agar) were prepared according to the manufacturer's instructions [13]

MacConkey Agar Preparation and inoculation

MacConkey agar is differential agar: 50 grams of MacConkey agar (powder) was added to 1 litre of deionized water and allowed to soak for 10 minutes. The Agar and deionized water were swirled to obtain a good mix and the mixture was thereafter sterilized by autoclaving for 15 minutes at 121^0C. The media was poured into petri-dishes after allowing it to cool to 47^0C. The culture plate was covered and allowed to set before inoculation of samples.

Chocolate Agar Preparation and inoculation
Chocolate agar is both an enriched and differential agar: 20 grams of chocolate agar (powder) was added to 1 liter of deionized water and allowed to soak for 10 minutes. The Agar and deionized water were swirled to obtain a good mix and the mixture was as well sterilized by autoclaving for 15 minutes at 121^0C. The media was poured into petri-dishes after allowing it to cool to 47^0C. The culture plate was then covered and allowed to set before inoculation of samples.

Inoculation, Incubation and culture reading
The swab samples were cultured aseptically on a highly nutritious non- selective media (chocolate and MacConkey agar) designed to support the growth of most commonly encountered bacteria and fungi. Cultures were then incubated in their culture plates at a temperature of 37^oc for 48 hours [14] in order to grow microorganism after which the culture plate was examined macroscopically against a bright light in order to grow microorganism.The culture plate was also examined macroscopically against a bright light in order to identify the isolated organisms based on their colonial characteristics using a light microscope. FIGURE 1 below shows the incubator machine used in incubating the organisms and FIGURE 2 shows the standard light microscope used in the study.

Data Analysis
Data were analyzed using Statistical Package for Social Science (SPSS) version 14.0 software. Descriptive statistics were applied in determining the frequency of occurrence. Chi- square test was applied to test the significance of site of collection and the type of organism isolated as well as time of collection and growth density. Results were tested for statistical significance at $p<0.05$.

RESULTS
Table 1 shows that Transabdominal probe and ultrasound gel harbored the highest percentage of staphylococcus aureus 46.2% (n=12) and 57.1% (n=4) respectively while ultrasound couch had the highest percentage of aerobic spore formers 31.4% (n=6). Table 2 shows that Transabdominal probe had growth density of 30.8% (n=8) in all. Transvaginal probe had scanty growth of 61.5% (n=8). From the ultrasound couch, 72.2% (n=13) of the samples had scanty growth, while 22.2% (n=4) had moderate growth, and no heavy growth was seen on the transvaginal probe. Table 3 shows that the percentage of some of the organisms isolated after scanning period was higher than that isolated before scanning period. Table 4 shows that heavy (40.0%; n=12) and moderate (26.7%; n=8) growth of organisms after scanning periods were quite higher than before scanning periods, while scanty growth (30.0%; n=9) was higher before scanning than after scanning periods, and 3.3% (n=1) of the sample were no growth after scanning period. Before scanning periods, scanty growth was 74.1% (n=20), moderate growth was 18.5% (n=5), there was no heavy growth seen and 7.4% (n=2) had no growth (table 4). The time of collection of the sample plays a significant role (p-0.002) in determining the density of organisms found and their growth density. Table 5 shows that staphylococcus aureus was the highest 33.8% (n=22). Other organisms isolated include aerobic spore formers (26.2%; n=17), staphylococcus epidermis (15.4 %; n=10), coliform (3.1%; n=2), candida albicans (6.2%; n=4), cladosporum spp (1.5%; n=1), pseudomonas aeruginosa (3.1%; n=2) and no growth observed (4.6%; n=3).

DISCUSSION

The use of ultrasonography in the characterization of intra-abdominal organs is well established [15,16]. It is an imaging modality that is quite easily available in urban Nigerian cities like Lagos. It is relatively less expensive and most people especially in urban areas now appear to be more aware of its importance in antenatal care as majority of ultrasound clinic attendees in the metropolitan Lagos are pregnant women. In spite of the status of Lagos as the commercial capital of Nigeria, it largely remains an urban slum where filth/poor sanitary condition is palpable. And with the global economic downturn, basic aseptic items such as alcohol- based sanitizers; antiseptic lotions and sometimes even running water are hard to come by in many hospital/diagnostic center. This picture is not different from what obtains in most countries in the Sub-Saharan Africa. It is therefore, not a surprise that the region is still grappling with preventable diseases and nosocomial infections [3,12]. Several investigators have reported that medical equipment, including ultrasound machines and transducers, may act as both a source and a vector of nosocomial infection [17,18]. It is also believed that under certain unfavorable circumstances ultrasound gel can become contaminated with a variety of pathogenic agents [17, 19,20]. Several studies have confirmed the transmission of bacteria and viruses from a patient's skin to ultrasound equipment, the most significant organisms including Staphylococcus aureus (including methicillin-resistant strains, i.e. MRSA), Pseudomonas, Acinetobacter species, Candida albicans, hepatitis (B and C), human immunodeficiency virus (HIV) and herpes [17,21]. The risk of infection associated with ultrasound transducers differs according to the nature of the examination for which they are used. The risk can be classified according to the system devised by Spaulding 40 years ago, as being non-critical, semi critical or critical [22,23]. Non-critical probes are those that come into contact with intact skin, as occurs during abdominal, breast and small-parts examinations. Semi-critical probes come into contact with a mucosal membrane, as occurs during transesophageal, transvaginal and transrectal ultrasonography, or diseased/breached skin. Critical probes come into contact with blood and/or other body cavity fluids or internal tissues, as occurs during intraoperative ultrasonography. Lagos metropolis, therefore, provides a unique environment to study ultrasound equipment as a possible vehicle for infection transmission especially during non-critical and semi-critical operations usually undertaken by most sonographers in the metropolis.

In the present study, a number of microorganisms were found on the transabdominal probe, transvaginal probe, ultrasound couch and the ultrasound gel. Such microorganisms isolated were aerobic spore formers, staphylococcus aureus, staphylococcus epidermidis, coliform, klebsiella pneumonia, Candida albicans, cladosporum spp. and pseudomonas aeruginosa. Staphylococcus aureus was the most commonly isolated microorganism (33.8%; n=22) from the ultrasound equipment. This finding support the opinion of Ohara et al [24] who had previously reported a high level of contamination of ultrasound equipment (39%) with staphylococcus aureus. Nester et al [25] did explain that staphylococcus aureus forms part of the skin’s natural flora and is found in up to 40% of healthy people. While we believe that this natural flora may have contributed to the percentage of staphylococcus aureus in our study, it must be highlighted that the microorganism is notorious for causing a number of illnesses. This according to Nester et al., [25] ranges from minor skin infection such as pimples, impetigo, boils (furuncle), cellulitis, scalded skin syndrome, and abscesses to life threatening diseases such as pneumonia, meningitis, pelvic inflammatory disease (PID), osteomyelitis, endocarditis and sepsis. Moreover, Nester et al [25] specifically reported that it is still one of the five major causes of nosocomial infection.
In the present study, staphylococcus epidermidis (23.1%) was also commonly isolated in the swabbed samples. This is similar to the findings of Abdullah [26] who found a 23.5% incidence of staphylococcus epidermidis in the ultrasound gel which has been recognized as a major infection associated with prosthetic joints and the urinary tract. Aerobic spore former was also commonly isolated (31.4%). Most of these isolated microorganisms exist naturally in the

environment and are ordinarily considered not highly pathogenic [18]. They could, however, cause nosocomial infections if favourable conditions such as diseased/breached skin and discharging sinuses are in a susceptible patient [18,23]. This implies, therefore, that sonographers in the metropolitan Lagos must adopt best practices in the disinfection of the ultrasound equipment and probe in order to prevent nosocomial infections.

It has been well documented that the transabdominal probe is a vehicle for infection transmission [24, 26]. Similar result was obtained in the present study as the most commonly contaminated ultrasound equipment was transabdominal probe with a heavy growth of microorganisms especially staphylococcus aureus (30.7%). This is also similar to the opinion of Mirza et al [27] who reported a high level of bacterial count on a transabdominal probe and suggested that such heavy growth was due to patient's body contact with the probe. While Ohara et al [24] reported the transmission of staphylococcus aureus and pseudomonas aeruginosa from patient's skin to the ultrasound probe, Spencer et al [26] also reported that transabdominal probe, if cultured after routine scanning of intact skin, may become colonized with skin flora in up to 30% of cases.

Transabdominal probe is the most frequently used probe for abdominal and pelvic ultrasonography in the Lagos metropolis during the period of the study. Sonographers in Lagos Nigeria must therefore adhere to manufacturer's recommendation to ensure that the transducer is cleaned and disinfected after each patient using the appropriate disinfectant [23]. In the present study, there was a statistical significant difference between site of collection of sample and growth density of microorganisms ($p=0.03$) whereas no statistical significant difference ($p>0.05$) was found between time of collection and density of bacterial growth. There was no statistical significant relationship between site of collection and organisms found in the study ($p=.498$) whereas as the site of collection played a significant role in growth density of bacteria ($p=0.03$). There was also no relationship between time of collection and isolated organism ($p=.418$). Time of collection played a significant role in determining the growth density of organisms found ($p=.0021$).These results seem to agree with Spaulding et al's [22] opinion that the risk of nosocomial infection depends on the nature of ultrasound examination (non-critical, semi-critical and critical). This suggests that semi-critical and critical ultrasound examinations may require higher level of disinfection in order to keep nosocomial infections at bay. Furthermore, it suggests that proper cleaning of the probe and the whole ultrasound equipment with the recommended disinfectant immediately after each patient and at the end of each day's work should be part of routine practice. Several investigators have assessed the potential of the coupling acoustic gel to act as a vehicle of cross contamination. For instance, outbreaks of both Klebsiella and Burkholderia6 infection have been reported within ultrasound departments and the source of infection traced back to the ultrasound gel [28,20].

An earlier study by Muradali et al [29] had previously shown that the gel could act as a culture medium and permitted bacterial growth. It was recommended by Muradali et al [29] that after the final ultrasound examination of the day, all transducers should be cleaned with a liquid cleaning solution such as chlorhexidine (0.05% weight/volume) to remove all traces of coupling gel which could otherwise support the overnight growth of bacteria. In the present study, ultrasound gel was also contaminated with organisms like staphylococcus aureus, aerobic spore former and pseudomonas aeruginosa (57.1%, n=4; 28.6%, n=2 and 14.3%). This agrees with earlier reports that showed that ultrasound gel is a vehicle for the spread of nosocomial infection [20]. In the present study, the ultrasound couch as well as the transvaginal probe was significantly contaminated with microorganisms. Reasons for the contamination of the couch may have included improper or no disinfection before and after scanning periods. Disinfection of the couch with a compatible disinfectant such as 70% alcohol solution or mechanical scrubbing with

alcohol-based sanitizers is recommended [20]. That the ultrasound couches were contaminated suggests that decontamination protocols were generally poor during the study period. Transvaginal probes, on the other hand, were also contaminated with such microorganisms as candida albicans and staphylococcus aureus. This result seems to support the report of Hignett and Claman's work [30] which suggested that all medium risk (semi-critical) ultrasound examinations carried out with endocavitary probes should be considered as high risk (critical) especially when there is a significant contamination by body fluids. In particular, Hignett and Claman [30] reported that 75-80% of covers used during endocavitary examinations are perforated. This implies that after endocavitary examination, care must be exercised to ensure that the probe and the surrounding are not contaminated with body fluids. A major limitation of this study appears to be that we were unable to categorically state the level of risk of nosocomial infection associated with ultrasonography as majority of examinations involve non-critical studies.

CONCLUSION

The ultrasound equipment as a possible vehicle for transmission of infection has been assessed. The ultrasound equipment, probe and ultrasound coupling gel all appear to present a significant risk for infection transmission even as staphylococcus aureus, staphylococcus epidermidis, aerobic spore formers, and klebsiella were major microorganisms found in the present study. It appears that extent of risk involved depends on the type of sonographic examination performed with patients who undergo endocavitary examinations appearing to be at a higher risk of contracting nosocomial infection. Sonographers in Lagos metropolis should, therefore, adhere to standard infection prevention protocol for all ultrasound examinations.

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Table 1a: The relationship between site of collection and isolate (organisms)

Isolated Organism	Site of collection							
	Trans-abdominal probe		Trans-vaginal probe		Ultrasound couch		Ultrasound Gel	
	n	%	n	%	n	%	n	%
No growth	2	7.7.*	0	0.0	1	5.3	9	0.0
Aerobic spore formers	6	23.1	3	23.1	6	31.4	2	28.6
Staph aureus	12	46.2	2	15.4	3	15.8	4	57.1
Staph epidemis	4	15.4	3	23.1	3	15.8	0	0.0
Coliform	0	0.0	1	7.6	1	5.3	0	0.0
Kleb pneumonia	0	0.0	2	15.4	2	10.5	0	0.0
Candida albicans	1	3.8	2	15.4	2	10.5	0	0.0
CladosporumSpp	0	0.0	0	0.0	1	5.3	0	0.0
Pseudomonas	1	3.8	0	0.0	0	0.0	7	14.3
Total	**26**	**100.0**	**13**	**100.0**	**19**	**100.0**	**22**	**100.0**

Table 1b: Chi square Tests

	Value	Df	P-value
Pearson	23.363	24	.498
N of valid cases	65		

Table 2a: The relationship between site of collection and growth density

Growth density	Site of collection							
	Trans-abdominal probe		Trans-vaginal probe		Ultrasound couch		Ultrasound Gel	
	n	%	n	%	n	%	n	%
No growth	2	7.6	0	0.0	1	5.6	0	0.0
Scanty growth	8	30.8	8	61.5	13	72.2	2	28.6
Moderate growth	8	30.0	5	38.5	4	22.2	4	57.1
Heavy growth	8	30.8	0	0.0	0	0.0	1	14.3
Total	26	100.0	13	100.0	18	100.0	7	100.0

Table 2b: Chi-square Tests

	Value	Df	P-value
Pearson	17.834	9	.037
N of valid cases	64		

Table 3a: The relationship between time of collection and isolated organisms

Isolated organism	Time of collection			
	Before Scanning		After scanning	
	n	%	n	%
No growth	2	7.1	1	3.3
Aerobic spore formers	8	28.6	7	23.3
Staph aureus	8	28.6	10	33.3
Staph Epidermidis	7	10.7	7	23.5
Coliform	2	7.1	0	0.0
Kleb pneumonia	1	3.6	3	10.0
Candida	3	10.7	1	3.3
CladosporumSpp	1	3.6	0	0.0
Pseudomonas Aeruginosa	0	0.0	1	3.3
Total	**28**	**100.0**	**30**	**100.0**

Table 3b: Chi square Tests

	Value	Df	P-value
Pearson N of valid cases	8.164 58	8	418

Table 4a: The relationship between time of collection and growth density

Isolated organism	Time of collection			
	Before Scanning		After scanning	
	n	%	n	%
No growth	2	7.4	1	3.3
Scanty growth	20	74.1	9	30.0
Moderate growth	518.5		8	26.7
Heavy growth	0	0.0	12	40.0
Total	27	100.0	30	100.0

Table 4b: Chi square Tests

	Value	Df	P-value
Pearson N of valid cases	15.273 57	3	.002

Table 5: Relationship between isolated organism and the frequency of occurrence of organisms.

Organisms isolated	Frequency of occurrence	
	n	%
No growth	3	4.6
Aerobic spore formers	17	26.2
Staph aureus	22	33.8
Staph Epidermidis	10	15.4
Coliform	2	3.1
Klebsiella pneumonia	4	6.2
Candida albicans	4	6.2
Cladosporum spp.	1	1.5
Pseudomonas Aeruginosa	2	3.1
Total	**65**	**100.0**

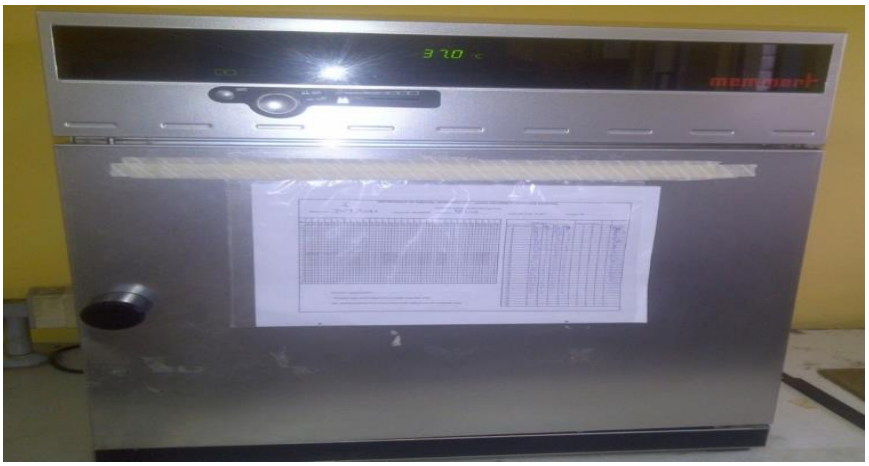

Figure 1: shows the incubator used for this process

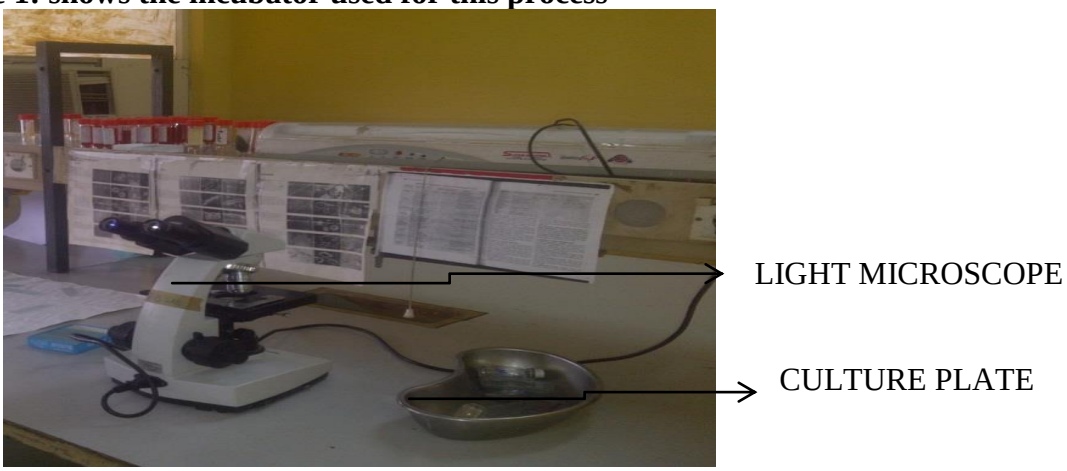

Figure 2: shows the microscope along with the culture plate used for isolation

HYBRID ALGORITHM FOR DOSE CALCULATION IN CMS XiO TREATMENT PLANNING SYSTEM

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ABSTRACT
Background: This study aimed at designing an improved hybrid algorithm by explicitly solving the linearized Boltzmann transport equation (LBTE) which is the governing equation that describes the macroscopic behaviour of radiation particles (neutrons, photons, electrons, etc). **Objective:** To develop an improved hybrid algorithm that has a good balance between speed and accuracy of calculation of dose distribution in in-homogeneous phantom. **Method:** The LBTE was solved numerically to compute photon transport in a medium. A programming code (algorithm) for the LBTE solution was developed and applied in the treatment planning system (TPS). The algorithm accuracy will be evaluated using a newly designed in-house verification phantom and its results will be compared to those of the other XiO photon algorithms. The accuracy of the algorithm was evaluated by creating several plans for both the designed phantom and solid water phantom using the designed algorithm and other Xio photon algorithms. The plans were sent to a pre-calibrated Eleckta linear accelerator for measurement of absorbed dose. **Result:** The results for all treatment plans using the hybrid algorithm compared to the 3 Xio photon algorithms were within 4 % limit. Calculation time for the hybrid algorithm was less in plans with larger number of beams compared to the other algorithms. **Conclusion:** The hybrid algorithm provides comparable accuracy in treatment planning conditions to the other algorithms. This algorithm can therefore be employed in the calculation of dose in advance techniques such as IMRT and Rapid Arc by a radiotherapy centres with cms xio treatment planning system as it is easy to implement.

Keywords: *Treatment Planning System, Algorithm, Photon beam, Dose, Phantom.*

INTRODUCTION

The pre-knowledge of radiation dose distribution in the patient is required undergoing radiotherapy treatment. This is determined by the simulation of the treatment procedure on a dedicated computerized system. Treatment planning can be described as the iterative process whereby the treatment strategy prescribed by the radiation oncologist. It is as a set of instructions, including the description of the expected dose distribution within the patient. Planning is based on predictions of dosage delivered to the patient by the proposed arrangement of radiation beams and the patient set-up. When a newly acquired treatment planning system (TPS) is being commissioned, beam data and other parameters from local linear accelerator must be entered into it. The data requirements for computerised TPS may be categorised in terms of data usage. The details and the format of the required data will depend on the type of each planning system. Patient treatment planning often requires both measured and user-determined data. It is necessary to verify that any averaging of data by the TPS algorithm does not produce unacceptable variations from the measured data. The efficiency of the calculation algorithm in both standard and non-standard conditions must be verified to ensure its suitability and limitations. Distinction must be made between the data collected for entry into the TPS and data collected in order to validate the TPS results. Basic data are used by the TPS to calculate dose distributions while reference data are acquired under standard conditions to validate results. After the commissioning process of the TPS, verification of its accuracy is often done using a

verification phantom, an object made of materials that mimic human body tissues, and that is treated like normal patient in the planning.

There are several algorithms in the TPS, each playing different roles. Dose calculation algorithms play the central role of calculating the dose within the patient using the beam parameters (Van Dyk, 1993). These algorithms comprise of a sequence of instructions that, with the set of input data, transform the information into a set of output results of interest to the user (Animesh, 2005). For every algorithm, the precision of the dose distribution depends on the data, the parameters used and the treatment conditions. Examples of commercially available algorithms includes, Anisotropic Analytic algorithm, Fast Fourier Transform Convolution algorithm, Superposition algorithm, Collapse Cone Convolution, Monte Carlo algorithm, Fast Superposition, Inhomogeneous Correction algorithm, Modified Clarkson Sector Integration, Area Integral algorithm etc. The accuracy with which algorithms are able to predict dose distribution is dependent upon the assumptions and approximations that the algorithms make. Also, the speed of calculation of Monitor Units (MU) is highly dependent on the number of fields in a treatment plan for most algorithms. The verification of the accuracy and the speed of these algorithms using heterogeneous phantom for measurement is important. An ideal algorithm is one which has good compromise between precision and speed in an inhomogeneous medium. Majority of the commercially available algorithms lack this quality and hence, the need for this study.

The CMS XiO treatment planning system which uses the Clarkson, Fast furrier Transform (FFT) convolution, Superposition, Faster Superposition, and Electron 3-D pencil beam algorithms are used for the calculation of photon and electron dose distributions. Each of the algorithms has its limitations regarding the balance between speed and precision, as such there is a need for an improved algorithm that can reduce the time of calculation with improved precision in dose calculation where multiple fields and larger MUs are involved. This is particularly essential for advanced radiotherapy techniques such as intensity modulated radiotherapy (IMRT) that involves many fields and large MUs.

METHODS

The Boltzmann Transport Equation (BTE) is the governing equation which describes the macroscopic behaviour of radiation particles such as photons, electrons, neutrons, protons etc. as they travel through and interact with matter. The Linear Boltzmann transport equation (LBTE) is a form of the BTE which assumes that radiation particles only interact with the matter and not with each other. This is valid under the conditions without external magnetic fields. There are different ways of solving the LBTE, however, the numerical methods proposed by Lewis et al. (1984) is the most suitable for solving the equation explicitly. The LTBE was solved using a similar method applied by Vassiliev et al. (2010):

$$\hat{\Omega}\cdot\vec{\nabla}\Psi^{y}+\sigma_t^{y}\Psi^{y}=q^{yy}+q^{y}, \quad \text{.....1}$$

$$\hat{\Omega}\cdot\vec{\nabla}\Psi^{e}+\sigma_t^{e}\Psi^{e}-\frac{\partial}{\partial E}(S_R\Psi^{e})=q^{ee}+q^{ye}+q^{e}, \quad \text{.....2}$$

The first terms on the left hand side of equations 1 and 2 are the streaming operators while the second terms are the collision or removal operators. Equation 2 is the Boltzmann Fokker- Planck transport equation (Wareing et al., 2000, Wareing et al., 2001), which is solved for the electron transport. In this equation the third term on the left hand side represents the continuous slowing down (CSD) operator, which accounts for Coulombic 'soft' electron collisions. The right hand side of Equations 1 and 2 include the scattering, production, and the external source terms (q^{y} and q^{e}). The scattering and production sources are defined by:

$$q^{yy}(\vec{r},E,\hat{\Omega}) = \int_0^{\infty} dE' \int_{4\pi} d\Omega' \sigma_s^{yy}(\vec{r},E' \rightarrow E, \hat{\Omega}\cdot\hat{\Omega}')\Psi^{y}(\vec{r},E',\hat{\Omega}'), \quad \text{.....3}$$

$$q^{ye}(\vec{r},E,\hat{\Omega}) = \int_0^{\infty} dE' \int_{4\pi} d\Omega' \sigma_s^{ye}(\vec{r},E' \rightarrow E, \hat{\Omega}\cdot\hat{\Omega}')\Psi^{y}(\vec{r},E',\hat{\Omega}'), \quad \textbf{.....4}$$

$$q^{ee}(\vec{r},E,\hat{\Omega}) = \int_0^{\infty} dE' \int_{4\pi} d\Omega' \sigma_s^{ee}(\vec{r},E' \rightarrow E, \hat{\Omega}\cdot\hat{\Omega}')\Psi^{e}(\vec{r},E',\hat{\Omega}'), \quad \textbf{.....5}$$

where

σ_s^{yy} = Macroscopic photon-to-photon differential scattering cross section

σ_s^{ye} = Macroscopic photon-to-electron differential production cross section

σ_s^{ee} = Macroscopic electron-to-electron differential scattering cross section

The following equation represents the un-collided photon fluence:

$$\hat{\Omega}\cdot\vec{\nabla}\Psi_{unc}^{y} + \sigma_t^{y}\Psi_{unc}^{y} = q^{y}(E,\hat{\Omega})\delta(\vec{r}-\vec{r}_p), \quad \text{.....6}$$

A property of Equation 6 was that $\vec{\nabla}\Psi_{unc}^{y}$ can be solved for analytically. Doing so provides the following expression for the un-collided photon angular fluence from a point source:

$$\Psi_{unc}^{y}(\vec{r},E,\hat{\Omega}) = \delta(\hat{\Omega}-\hat{\Omega}_{\vec{r},\vec{r}_p})\frac{q^{y}(E,\hat{\Omega})}{4\pi}\frac{e^{-\tau(\vec{r},\vec{r}_p)}}{\left|\vec{r}-\vec{r}_p\right|^2}, \quad \text{.....7}$$

where,

$\hat{\Omega}_{\vec{r},\vec{r}_p} = \dfrac{\left|\vec{r}_p-\vec{r}\right|}{\left|\vec{r}-\vec{r}_p\right|}$ and $\vec{r}_p$ and $\vec{r}$ are the source and destination points of the ray trace, respectively. $\tau(\vec{r}-\vec{r}_p)$ is the optical distance measured in mean-free-paths between $\vec{r}$ and $\vec{r}_p$.

Once the electron angular fluence was solved for all energy groups, the dose in any output grid voxel was obtained through the following equation proposed by Siebers et al. (2000)

$$D_i = \int_0^{\infty} dE \int_{4\pi} d\hat{\Omega} \frac{\sigma_{ED}^{e}(\vec{r},E)}{\rho(\vec{r})}\Psi^{e}(\vec{r},E,\vec{\Omega}), \quad \text{.....8}$$

where, σ_{ED}^{e} = macroscopic electron energy deposition cross sections (in MeV/cm)

$\rho =$ Material density (in g/cm^3)

Figure 1 show the iteration scheme used to solve the equation.

Determination of absorbed dose using the hybrid algorithm and other CMS XiO algorithms

Treatment plans were designed to prescribe 1.0 Gy at the linear accelerator iso-centre using the hybrid and other CMS XiO algorithms. The calculation speeds for absorbed dose with the different algorithms were determined for several plans. The plans were transferred to the pre-calibrated Elekta-Precise clinical linear accelerator (Eleckta Oncology System, 2000) for applications. Measurements were carried out with 6 MeV photon beams using an iso-centric set-up as shown in figures 2a and b respectively. A pre-calibrated Farmer-type ionization chamber along with its electrometer (figure 3) was used to determine the absorbed dose delivered. The ionization chamber was cross calibrated against a reference ionization chamber in order to obtain the calibration factor. Measurements were made at the depth of 12 cm in the solid water, which corresponds to the position of the ionisation chamber in the phantom. Six measurements were

made for each treatment plan using the different algorithms for comparison. The absorbed dose at the reference depth was determined using the dose to water IAEA TRS 398 protocol (IAEA, 2000).

The absorbed dose at reference depth was calculated using the relation:

$D_{w,Q} = M_Q \times N_{D,w,} \times k_{Q,Qo}$ ……..…… (9)

where M_Q is the electrometer response corrected for temperature and pressure.

$N_{,D,w}$ is the chamber calibration factor and $k_{Q,Qo}$ is the factor which corrects for the difference in the response of the dosimeter at the calibration radiation quality Q_o and at quality Q of the clinical x-ray beam according to the IAEA TRS 398 protocol (IAEA, 2000). Calculated dose at D_{max} was compared to the expected dose of 1 Gy. Deviation between expected and measured dose was obtained using the relation:

$$\% \text{ Deviation} = \frac{D_{meas} - D_{ref}}{D_{ref}} \times 100 \quad \text{.....(10)}$$

RESULTS

The result of the absorbed dose measured at the LINAC for 6 MeV photon beam using the different algorithms are presented in appendix I. Figure 4 and 5 shows the results of the times used by the different algorithms for calculation of monitor units needed to deliver the prescribed dose for different plans at the 6 and 18 MeV photon beam.

DISCUSSION AND CONCLUSION

Table 1 shows the results of the absorbed dose measured in solid water along with the deviation from the reference dose and the standard deviation between the measurements taken for the 6 MeV photon beam. FSS and HB showed better accuracy (1 % dev.) in tables 1(a) and (b). The C algorithm had better accuracy (0 % dev.) in the 12-field plan shown in tables 1(c).Table 2 shows results of the absorbed dose measured for different field plans with the bone inhomogeneity along with the percentage deviation from the reference dose and the standard deviation for the 3 measurements taken for the 6 MeV photon beams. In tables 2(a) and (c), results of HB and FSS algorithms showed better accuracy (1 and 0 % dev. respectively) compared to others while convolution (C) showed the least accuracy (4 % dev.). HB was the only algorithm that showed improved accuracy (2 % dev.) in the wedged field according to table 2(b), while S along with HB had the best improved accuracy (0 and 1 % dev.) in the 12-field plans as shown in table 2(c). Table 3 shows the results of the absorbed dose measured with the lung inhomogeneity along with the percentage deviation from the reference dose and the standard deviation between the 3 measurements taken for the 6 MeV photon beam. FSS and HB showed better accuracy (1 % dev.) in tables 3(a) and (b) compared to C and S. S and HB had a better accuracy (0 and 1 % dev.) in the 12-field plan as shown in table 3 (c).

The results for all plans using the 4 algorithms in both beams were within the established limits (Van Dyk et al., 1993, Ahnesjo and Aspradakis, 1999, Fraass et al., 1998) and follow similar trend as those of Butts et al. (2001) where anthropomorphic phantom was used. The method and results of the hybrid algorithm also follow similar pattern as those of Kelly (2011) where the LBTE was used to compute neutron transport equation. Larger deviations observed with the convolution algorithm with the bone could be due to unaccounted for scattered radiation contribution from the inhomogeneous material by the algorithm (Animesh, 2005. Muralidhar, 2009). However, convolution is good where there are no inhomogeneities as seen in tables 1 and 4. There is a general improvement across the tables for all algorithms in the larger field plans while poor deviation is noticeable for the wedged field plans across board. This may be due to the inability of the algorithms to model the fluence calculation for wedges (Van Dyk et al., 1993. Van Dyk et al., 1997). There is a similar trend in the results of the FSS and S, this may be due to

the similarity in the methods (collapse cone) both algorithms use for calculation. Other sources of uncertainty such as set-up, phantom and the detector could have as well contributed to the deviation.

Calculation time in a single or fewer fields are longer with the hybrid algorithm than the convolution and superposition as shown in figures 4 and 5. Larger fields and higher energies take longer time to calculate, as phantoms containing larger amount of bones as observed in figure 5 for the 18 MeV photon beam results. Most of the hybrid calculation time is spent in solving for the scattered photon and electron fluencies, which are performed only once for all beams in a treatment plan. As a result, hybrid calculation time scale varies weakly with the number of fields. However, convolution and superposition calculation time increases linearly with the number of fields. As a result, the relative calculation speed of the hybrid increases with increasing number of fields. In cases with larger number of fields (i.e., 6-, 9- and 12-field plans as it is the case in IMRT, Rapid Arc etc), Hybrid becomes significantly faster than other algorithms. The hybrid algorithm showed general improvement across the board in all the treatment plans and since it can be used with the original data requirements of the XiO treatment planning, no extra data are needed for its implementation.

The hybrid dose calculation algorithm was developed to address the accuracy and speed requirements for modern techniques in radiation therapy including IMRT and Rapid Arc. This hybrid algorithm provides comparable accuracy in treatment planning to standard algorithms such as the convolution, superposition and fast superposition as shown in the results. Validation has been performed to ensure dose calculation precision in typical inhomogeneous phantom. This algorithm can therefore be employed in the calculation of dose in advanced techniques such as IMRT and Rapid Arc by radiotherapy Centres with multiple algorithm system because it is easy to implement.

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APPENDIX I

Table 1: Comparison of absorbed doses (Gy) in solid water phantom for (a) Single field (b) Wedged field and (c) 12 fields for different algorithms.

(a)

	SOLID WATER			
	SINGLE FIELD			
	C	FSS	S	HB
	1.03	1.03	1.03	1.01
	1.03	1.02	1.03	1.01
	1.03	1.02	1.02	1.01
Average	1.03	1.02	1.03	1.01
STD	0.004	0.006	0.004	0.005
%Dev	3	2	3	1

(b)

	SOLID WATER			
	WEDGE FIELD			
	C	FSS	S	HB
	1.03	1.03	1.03	1.01
	1.03	1.02	1.03	1.01
	1.03	1.02	1.02	1.01
Average	1.03	1.02	1.03	1.01
STD	0.004	0.006	0.004	0.004
%Dev	3	2	3	1

(c)

	SOLID WATER			
	12 FIELDS PLAN			
	C	FSS	S	HB
	1.01	1.01	1	1
	1	1.01	1	1.01
	1	1.01	1	1.01
Average	1	1.01	1	1.01
STD	0.004	0	0.004	0.005
%Dev	0	1	1	1

Table 2: Comparison of absorbed doses in bone for different field plans, showing percentage deviation from reference dose (1.00Gy)for (a) Single field (b) Wedged field and (c) 12 fields for different algorithms.

(a)

	BONE			
	SINGLE FIELD			
	C	FSS	S	HB
	1.04	1.01	1.02	1
	1.03	1.01	1.02	1
	1.04	1.01	1.02	1
Average	1.04	1.01	1.02	1
STD	0.004	0.005	0	0.004
%Dev	4	1	2	0

(b)

	BONE			
	WEDGE FIELDS			
	C	FSS	S	HB
	1.04	1.03	1.03	1.02
	1.04	1.03	1.03	1.02
	1.04	1.03	1.03	1
AVERAGE	1.04	1.03	1.03	1.02
STD	0	0.004	0	0.008
%Dev	4	3	3	2

(c)

	BONE			
	12 FIELDS			
	C	FSS	S	HB
	1.01	1.01	1.01	1
	1.01	1.01	1	1
	1.01	1.01	1	1
Average	1.01	1.01	1	1
STD	0	0.004	0.004	0
%Dev	1	1	0	0

Table 3: Comparison of absorbed doses in lung for different field plans, showing percentage deviation from reference dose (1.00Gy)for (a) Single field (b) Wedged field and (c) 12 fields for different algorithms.

(a)

	LUNG			
	SINGLE FIELD			
	C	FSS	S	HB
	1.02	1.01	1.02	1.01
	1.02	1.01	1.02	1
	1.02	1.01	1.02	1
AVERAGE	1.02	1.01	1.02	1.01
STD	0.004	0.004	0.004	0.005
%Dev	2	1	2	1

(b)

	LUNG			
	WEDGE FIELDS			
	C	FSS	S	HB
	1.02	1.01	1.02	1.01
	1.02	1.01	1.02	1.01
	1.02	1.01	1.02	1.01
AVERAGE	1.02	1.01	1.02	1.01
STD	0	0.004	0	0.006
%Dev	2	1	2	1

(c)

	LUNG			
	12 FIELDS PLAN			
	C	FSS	S	HB
	1.01	1.01	1	1
	1.01	1.01	1.01	1
	1	1.01	1	1
AVERAGE	1.01	1.01	1	1
STD	0.004	0.004	0.004	0.004
%Dev	1	1	0	0

```
% File: Linear Boltzmann Equations
% Date: 12th of March 2012
% Author: Michael Akpochafor
% the equation here perform time independent single calculation at high resolutions
% D (vector (r)) =\int(mu/P)*\(psi)_p{vector (r)'*A*[vector(r)-vector(r)']*d^3 *(vector
(r)')}
% D(vector (r))=dose at a point
%(mu/P)=mass attenuation coefficient
%\(psi)_p{vector (r)'=primary photon energy fluence
%A*[vector(r)-vector(r)']=convolution kernel, the distribution of fraction energy Imparted
per unit volume.
% (vector (r)')=TERMA at depth includes the energy retained by the photon.
% Plots a Linearized Boltzmann distribution Equations
% for dose calculation.
% THIS PROGRAMME SOLVE THE EQUATION (8)
% D (i)=int_0 ^inffy *dE*int_4*pi ^inffy *d(\omega)vector *\frac \sigma_ED
% ^e (r(vector), E)/\rho(vector)*r(vector)*\psi^e (r,E,\omega(all vector))
endTime = 5000;
tlist = 0:50:endTime;
numNodes = size(p,2);
% Set the initial temperature of all nodes to ambient, 300 K
u0 (1:numNodes) = 300;
% Find all nodes along the bottom edge and set their initial temperature
% to the value of the constant BC, 1000 K
nodesY0 = abs(p(2,:)) < 1.0e-5;
u0 (nodesY0) = 1000;
rtol = 1.0e-3; atol = 1.0e-4;
% The transient solver parabolic automatically handles both linear
% and nonlinear problems, such as this one.
u = parabolic(u0, tlist, b,p,e,t,c,a,f,d,rtol,atol);
figure;
plot (tlist, u(3, :)); grid;
title 'Temperature Along the Top Edge of the Plate as a Function of Time'
xlabel 'Time, seconds'
ylabel 'Temperature, degrees-Kelvin'
%figure;
pdeplot(p, e, t, 'xydata', u(:,end), 'contour', 'on', 'colormap', 'jet');
title(sprintf('Temperature In The Plate, Transient Solution( %d seconds)\n', ...
tlist(1,end)));
xlabel 'X-coordinate, meters'
ylabel 'Y-coordinate, meters'
% fprintf('\nTemperature at the top edge of the plate(t=%5.1f secs) = %5.1f degrees-K\n', ...
tlist (1,end), u(4,end));
```

Figure 1: The iteration scheme used to solve the equations.

Fig 2a: Isocentric set up with the designed phantom

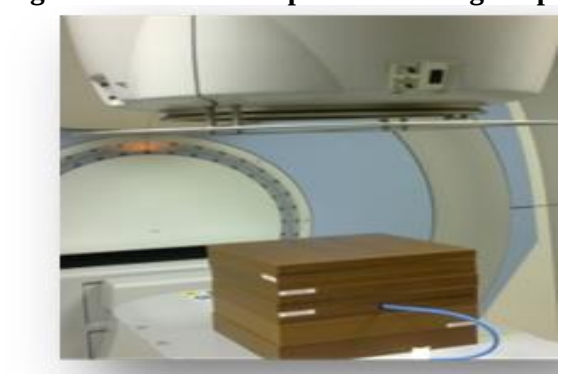

Fig. 2 b: The solid water phantom set-up under linear accelerator

Fig. 3: Farmer type ionization chamber along with its electrometer.

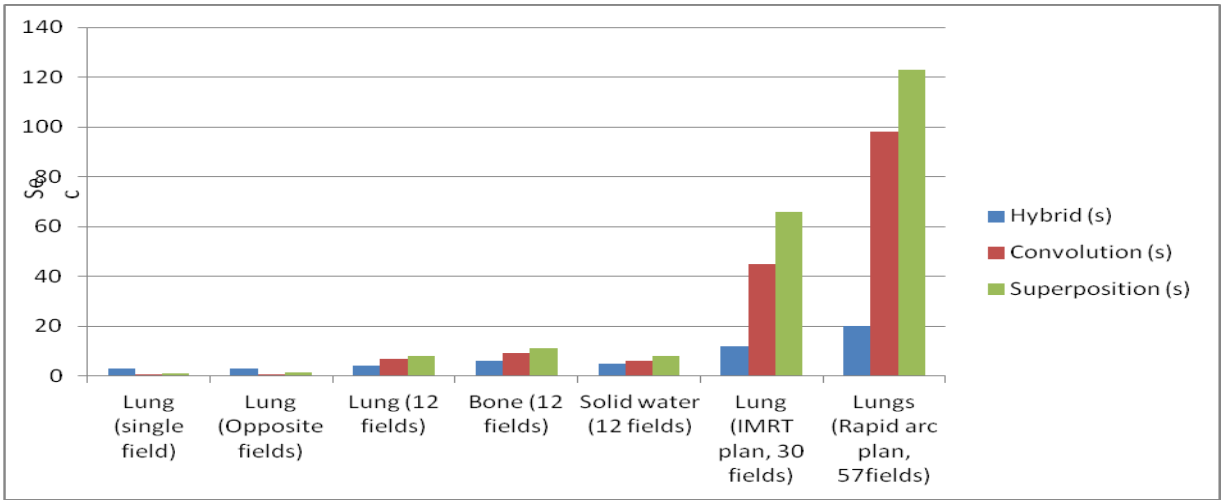

Figure 4: Speed of the different algorithms for different treatment plans for 6 MeV photon beam.

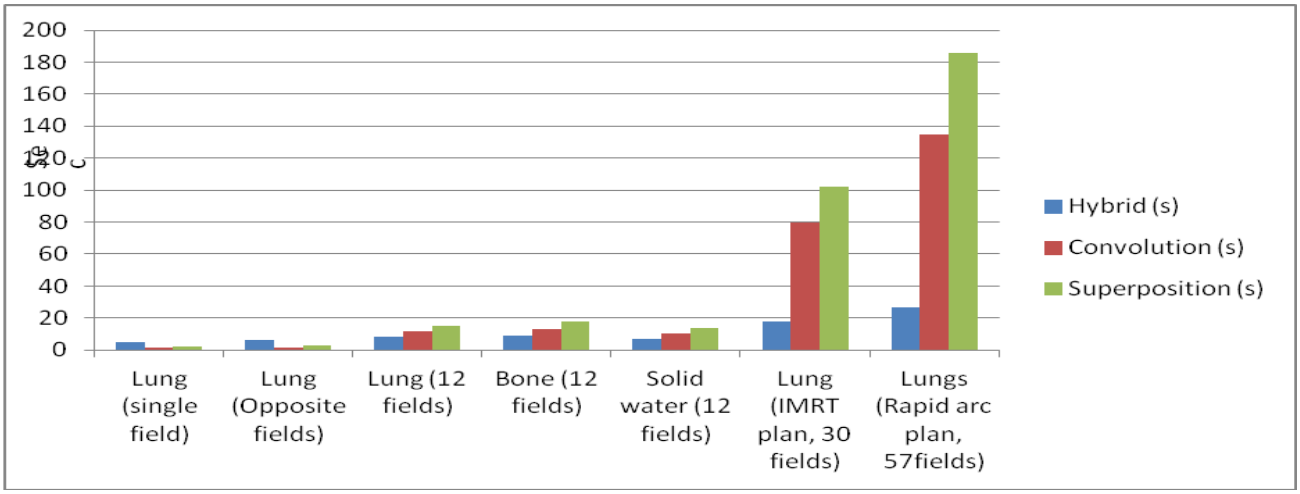

Figure5: Speed of the different algorithms for different treatment plans with 18 MeV photon beam.

SIMULATION OF THE LINEAR BOLTZMANN TRANSPORT EQUATION IN MODELLING OF PHOTON BEAM DATA

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ABSTRACT
Background: A beam data modeling algorithm was design by solving the linear Boltzmann transport equation. The Linear Boltzmann transport equation (LBTE) is a form of the Boltzmann transport equation that assumes that radiation particles only interact with the matter they are passing through and not with each other.This condition is only valid when there are no external magnetic fields. **Objective:** To develop a beam data modeling algorithm that is capable of computing electron contamination component of photon beam. **Method:** The numerical method proposed by Lewis et al., [9] was used to solve the LBTE. A programming code was computed for the LBTE and run on cms xio treatment planning system to generate beam data, the generated beam data were compared to experimentally measured data and analyzed. **Results:** The calculated PDDs completely overlaps the measured PDDs for the small field sizes while there is a shift in the PDD tail for large field size. However the shift is negligible. For the wedge PDDs, the shift between the measured PDDs and the calculated occurs at the Dmax region and it increases with increase in field size. The calculated wedge profiles have a slight shift at the shoulder compared to the measured ones and this decreases with increase in field size unlike the PDDs. There is also a slight shift between calculated inplane profiles and measured ones. There is a good agreement between the measured beam data and the calculated ones using the algorithm. **Conclusion:** This algorithm can be implemented as an in-house algorithm for beam data modeling and also as an independent quality assurance tool for checking the accuracy of clinical TPS algorithms with regards to beam data modeling during quality assurance checks and commissioning.

Keywords: *Treatment Planning System, Algorithm, Photon Beam data, Dose, Phantom.*

INTRODUCTION

Computerized TPS are used in external beam radiotherapy to simulate beam shapes and dose distribution with the intent to maximize tumour control and minimize normal complications [7]. Treatment simulations are used to plan the geometric and radiological aspects of the treatment using radiation transport and optimization principles. TPS facilitate prescribed dose delivery in which a number of parameters of the patient and of the tumour have to be taken into consideration such as the shape, size, depth etc. TPS commissioning involves the entry of beam data measured at the linear accelerator into the TPS machine and the accurate modelling of these beam data. Beam profiles and Percentage depth doses (PDD) are some of the most important beam data required for the commissioning of the TPS. The profiles and the PDD combine to form the isodose curve which determines the dose distribution in the plan of a radiotherapy patient. The profiles tails also determine the penumbra region of the dose distribution which plays a significant role in the total dose distribution. An improvement in the penumbra region of the profile will lead to a better dose distribution. The modelling of the beam data is done using the TPS software. The accuracy of the model depends on the software parameters and the3 measured data. There are several algorithms in the TPS software that play different roles, however the dose calculation algorithms play the central role of calculating dose distribution within the target volume at any given point [1]. Algorithms are a sequence of instructions that

operate on a set of input patient and dosimetric data, transforming the information into a set of desired output results [8]. For every algorithm, the precision of the dose calculation depends on the input parameters used. Different types of dose calculation algorithms are used in modern TPSs. The early TPS calculation methods were based on tabular representation of the dose distribution obtained directly from beam measurements. As time goes by, calculation models become more sophisticated as computer power grows, TPS calculation algorithms progressively matures towards more physically based models. The most advanced current algorithms are based on the Monte Carlo approach where the histories of many millions of photons are traced as they interact with matter using basic physics interactions. There is a full range of possibilities between table based models and Monte Carlo models. For every algorithm, the quality of the dose calculation is strongly dependent on the data or parameters used by the algorithm and its accuracy to predict dose rely on the assumptions and approximations that the algorithm makes. The type and quantity of the data needed varies according to the model. Usually, for measurement based models a lot of tables are required, whereas for physical based models only some parameters are necessary. Good understanding of the algorithms used within the TPS can help the user understand the strengths and limitations of the specific algorithms. This can also help the user diagnose TPS problems and develop a QA protocol.

It is important to understand the general principles of the model and its implementation details. The model parameters and input data have a significant impact on the accuracy of the calculation results. Even if the model is able to account for a given physical effect, the actual implementation in the treatment planning software is often simplified, leading to inaccurate or unexpected results for certain situations. Because of these situations an independent way of checking the algorithms accuracy with regards to beam data modelling is vital to achieve a proper QA exercise. Following the acceptance and commissioning tests of a computerized TPS, a quality assurance program should be established to verify the performance of the system. Several ways of carrying out the quality assurance of TPS has been proposed by various authors [1-6]. However, it is necessary that each Department develop its own program based on the availability of relevant equipment and according to local requirements, while using standard methods as guideline. In this study, the linear Boltzmann transport equation (LBTE) was solved following the numerical methods described by Lewis et al., [9], a programming code was computed for the LBTE and run on a MATLAB computing system to generate beam data (profiles and PDD), the generated beam data were compared to experimentally measured data and analysed.

METHODS

The Boltzmann transport equation (BTE) is the governing equation which describes the macroscopic behaviour of radiation particles (photons, electrons, neutrons, protons, etc.) as they travel through and interact with matter. The Linear Boltzmann transport equation (LBTE) is a form of the BTE which assumes that radiation particles only interact with the matter they are passing through and not with each other: this is valid for conditions without external magnetic fields. There are different ways of solving the LBTE; however, the numerical method proposed by Lewis et al., [9] is the method that can be used to solve the equation explicitly.

The LTBE was solved using a similar method applied by Vassiliev et al [15]:

$$\hat{\Omega}\cdot\vec{\nabla}\Psi^{y}+\sigma_t^{y}\Psi^{y}=q^{yy}+q^{y}, \quad \text{.....1}$$

$$\hat{\Omega}\cdot\vec{\nabla}\Psi^{e}+\sigma_t^{e}\Psi^{e}-\frac{\partial}{\partial E}(S_R\Psi^{e})=q^{ee}+q^{ye}+q^{e}, \quad \text{.....2}$$

Where,

Ψ^{y} = Angular photon fluence (or fluence if not time integrated), $\Psi^{y}(\vec{r},E,\hat{\Omega})$, is a function of position $\vec{r}$ =(x,y,z), energy E, and direction $\hat{\Omega}$=(μ,η,ζ)

Ψ^{e} = Angular electron fluence, $\Psi^{\gamma}(\vec{r},E,\hat{\Omega})$

q^{yy} = Photon – to – photon scattering source, $q^{yy}(\vec{r},E,\hat{\Omega})$, resulting from photon interactions.

q^{ee} = Election – to – electron scattering source, $q^{ee}(\vec{r},E,\hat{\Omega})$, resulting from electron interactions.

q^{ye} = Photon – to - electron scattering source, $q^{ye}(\vec{r},E,\hat{\Omega})$, resulting from electron interactions.

q^{y} = Extraneous photon source, $q^{y}(E,\hat{\Omega})$, for point source ***P,*** at position $\vec{r}_p$

This source represents all photons coming from the machine source model (Wareing et al., 2000),.

q^{e} = Extraneous electron source, $q^{y}(E,\hat{\Omega})$, for point source ***P,*** as position $\vec{r}_p$

This source represents all electrons coming from the machine source model.

$\sigma_t^{y} = \sigma_t^{y}(\vec{r},E)$ is the macroscopic photon total cross section in cm^{-1}

$\sigma_t^{e} = \sigma_t^{e}(\vec{r},E)$ is the macroscopic electron total cross section in cm^{-1}

$\sigma_t = \sigma_t(\vec{r},E)$, is the macroscopic total cross section in cm^{-1}

$S_R = S_R(\vec{r},E)$ is the restricted collisional plus radiative stopping power,

The first term on the left hand side of equations 1 and 2 is the streaming operator. The second term on the left hand side of equations 1 and 2 is the collision or removal operator. Equation 2 is the Boltzmann Fokker- Planck transport equation [11-12], which is solved for the electron transport. In Equation 2, the third term on the left represents the continuous slowing down (CSD) operator, which accounts for Coulomb 'soft' electron collisions. The right hand side of Equations 1 and 2 include the scattering, production, and the external source terms (q^{y} and q^{e}). The scattering and production sources are defined by:

$$q^{yy}(\vec{r},E,\hat{\Omega}),=\int_0^{\infty}dE'\int_{4\pi}d\Omega'\sigma_s^{yy}(\vec{r},E'\rightarrow E,\hat{\Omega}\cdot\hat{\Omega}')\Psi^{y}(\vec{r},E',\hat{\Omega}'),\quad\text{.....3}$$

$$q^{ye}(\vec{r},E,\hat{\Omega}),=\int_0^{\infty}dE'\int_{4\pi}d\Omega'\sigma_s^{ye}(\vec{r},E'\rightarrow E,\hat{\Omega}\cdot\hat{\Omega}')\Psi^{y}(\vec{r},E',\hat{\Omega}'),\quad\text{.....4}$$

$$q^{ee}(\vec{r},E,\hat{\Omega}),=\int_0^{\infty}dE'\int_{4\pi}d\Omega'\sigma_s^{ee}(\vec{r},E'\rightarrow E,\hat{\Omega}\cdot\hat{\Omega}')\Psi^{e}(\vec{r},E',\hat{\Omega}'),\quad\text{.....5}$$

Where

σ_s^{yy} = Macroscopic photon-to-photon differencial scattering cross section

σ_s^{ye} = Macroscopic photon-to-electron differencial production cross section

σ_s^{ee} = Macroscopic electron-to-electron differencial scattering cross section

The following equation represents the un-collided photon fluence:

$$\hat{\Omega}\cdot\vec{\nabla}\Psi^{y}_{unc}+\sigma^{y}_{t}\Psi^{y}_{unc}=q^{y}(E,\hat{\Omega})\delta(\vec{r}-\vec{r}_{p}),\quad\text{.....6}$$

A property of Equation 6 was that $\vec{\nabla}\Psi^{y}_{unc}$ can be solved for analytically. Doing so provides the following expression for the un-collided photon angular fluence from a point source:

$$\Psi^{y}_{unc}(\vec{r},E,\hat{\Omega})=\delta(\hat{\Omega}-\hat{\Omega}_{\vec{r},\vec{r}_{p}})\frac{q^{y}(E,\hat{\Omega})}{4\pi}\frac{e^{-\tau(\vec{r},\vec{r}_{p})}}{\left|\vec{r}-\vec{r}_{p}\right|^{2}},\quad\text{.....7}$$

where,

$\hat{\Omega}_{\vec{r},\vec{r}_{p}}=\dfrac{\left|\vec{r}_{p}-\vec{r}\right|}{\left|\vec{r}-\vec{r}_{p}\right|}$, and $\vec{r}_{p}$ and $\vec{r}$ are the source and destination points of the ray trace, respectively.

$\tau(\vec{r}-\vec{r}_{p})$ = The optical distance (measured in mean-free-paths) between $\vec{r}$ and $\vec{r}_{p}$.

Once the electron angular fluence was solved for all energy groups, the dose in any output grid voxel was obtained through the following equation proposed by Siebers et al. [13]:

$$D_{i}=\int_{0}^{\infty}dE\int_{4\pi}d\hat{\Omega}\frac{\sigma^{e}_{ED}(\vec{r},E)}{\rho(\vec{r})}\Psi^{e}(\vec{r},E,\vec{\Omega}),\quad\text{.....8}$$

Where,

σ^{e}_{ED} = macroscopic electron energy deposition cross sections (in MeV/cm)
ρ = Material density (in g/cm^3)

Experimental measurement of profiles and PDDs

A pre-calibrated Eleckta precise clinical linear accelerator was used to collect the beam data (profile and PDD). The profile and the PDD data were collected by following the guideline recommended by the Xio beam modelling guide [14]. The diagonal Profile Scans were collected at an SSD of 100cm with the Largest Open Field size 40x40 cm^2 and at various depths of 0.5, 1.0, 2.0, 3.0, 5.0, 10.0, 20.0, 30.0, up to the deepest obtainable depth. Scans were generated at an increment depth of 3 mm. The open field profiles were collected for the square field sizes of 5x5, 10x10, 15x15, 20x20, 25x25, 30x30 cm^2 and at depths of dmax, 5.0, 10.0, 20.0, and 30.0 cm. Scans were made in the in-plane direction for unrotated collimator. Scans were made at an increment depth of 2mm. Wedged aligned profile scans were collected in the wedged direction for the square field sizes of 10x10, 20x20 cm^2 at depths dmax, 5.0, 10.0, and 20.0 cm. Scans were made at an increment depth of 2 mm. The open Field PDDs were measured at the Field Sizes of 3x3, 4x4, 5x5, 7x7, 10x10, 12x12, 15x15, 20x20, 25x25, 30x30, 35x35,40x40 cm^2. The scans were made at an increment depth of 1 mm up to the deepest obtainable depth (35 cm). The wedge Field PDDs were measured at the Field Sizes of 5x5, 10x10, 20x20 cm^2. Scans were also acquired at an increment depth of 1 mm up to the deepest obtainable depth (35 cm). Once all scans were acquired for both 6 and 18 MV photon beam, they were compared to the computed

ones using the algorithm above and analysed. The experimental set up for the measurement is shown in figure 1 below.

RESULTS

Scanned data for 6 MV photon beam

Below are the results of the measured vs calculated PDDs and profiles. The coloured lines (—) represents the calculated PDDs and profiles while the black line (—) represents the measured ones.

DISCUSSION AND CONCLUSION

The results of the normal PDDs determined at reference depth of 10cm for different field sizes against the calculated PDDs for the 6 MV photon beam are represented in fig.2a and b. The calculated PDDs completely overlaps the measured PDD as observed in fig.2a for the small field sizes while there is a shift in the PDD tail for large field sizes as observed in fig. 2b. However the shift is negligible. For the wedge PDDs, the shift between the measured PDDs and the calculated occurs at the Dmax region and it increases with increase in field size as observed in fig 3a and b. This may be due to inability of the algorithm to model the fluence calculation for wedge [7-8]. The results of the normal PPDs for the 18 MV photon beam follows a similar pattern to those of the 6 MV photon beam. Electron contamination has been shown to increase in larger field sizes and higher photon energy [10], this is evident in the result of the 18 MeV photon beam presented in fig. 6 a and b. The electron contamination in the smaller field size (3 x 3 cm^2) PDD in fig. 6a is much lesser compared to that of the 12 x 12 cm^2 PDD, this is because electron contamination is mostly caused by the components (flattening filter, collimators, monitor chamber, etc) in the head of the LINAC. When collimator opening is decrease (i.e. small field size), the electron contamination also decreases as part of the electron source would have been shielded by the collimator blocks.

The variation of dose occurring on a line perpendicular to the central beam axis at a certain depth is known as the beam profile. It represents how dose is altered at points away from the central beam axis. The calculated wedge profile for the 6 MeV photon beam have a slight shift at the shoulder as observed in figures 4a compared to the measured ones and this decreases with increase in field size unlike the PDDs. There is also a slight shift between calculated inplane profiles as shown in figures 5a. The 18 MV photon beam inplane profiles follows similar pattern to those of the 6 MeV photon beam. However, large deviations between calculated and measured are observed in the profiles of the larger field sizes (30 x 30 cm^2 and 40 x 40 cm^2), this is of less concern since most clinical field sizes are lesser. Generally, there is an improvement in the tail region of all the calculated profiles; the region that determines the penumbra of the beam. The penumbra is the region of rapid dose fall off located at the edge of a beam. It is usually considered to be the part of the dose that lies between 20 and 80 % of the central axis dose. The slight shifts between the calculated and the measured PDDs and profiles are negligible.

Overall, there is a good agreement between the measured beam data and the calculated ones as shown in the results using the algorithm. This algorithm can be implemented as an in-house quality assurance tool for checking the accuracy of clinical TPS algorithms with regards to beam data modelling during commissioning and annual QA checks.

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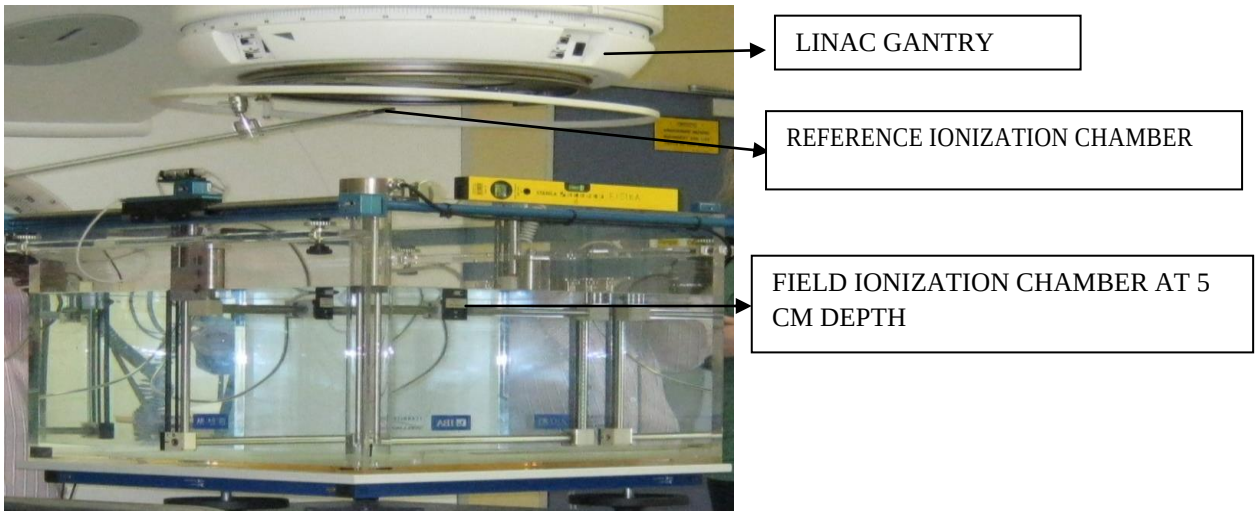

Fig. 1: Experimental set up for acquisition of beam profile and PDD scans

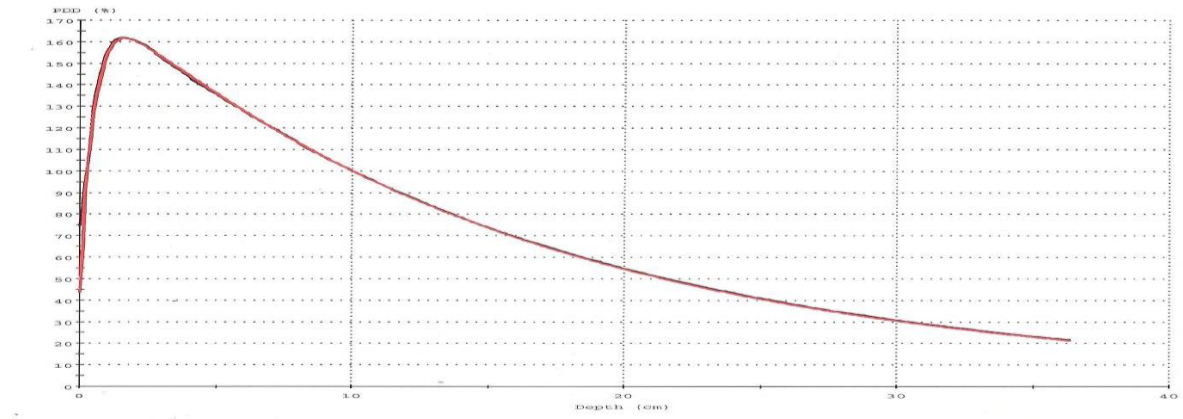

Fig. 2a: normal PDD for 3 x 3 cm^2 field size

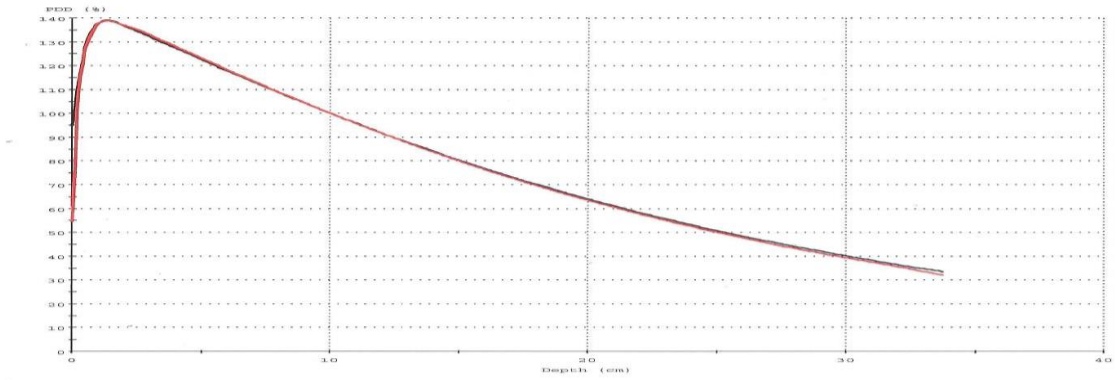

Fig. 2 : normal PDD for 40 x 40 cm^2 field size

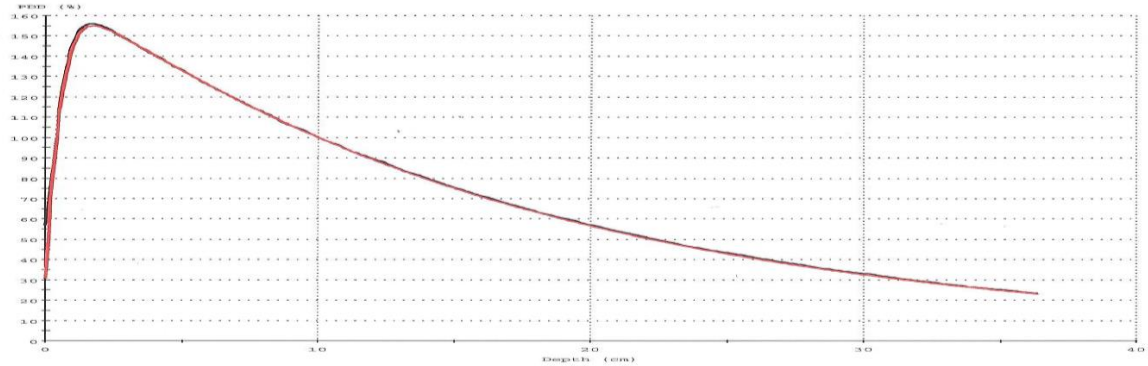

Fig. 3 a: wedge PDD for 3 x 3 cm^2 field size

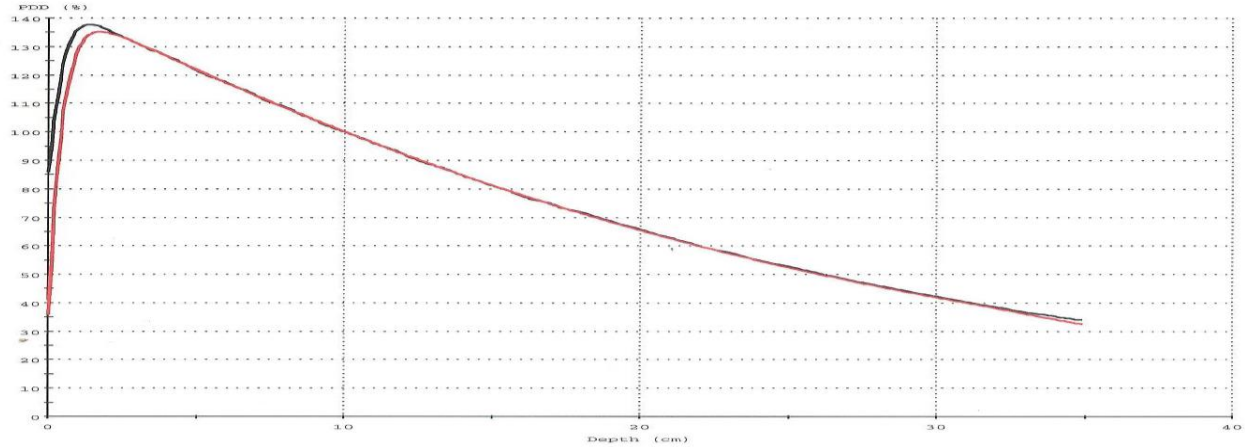

Fig. b: wedge PDD for 30 x 30 cm^2 field size

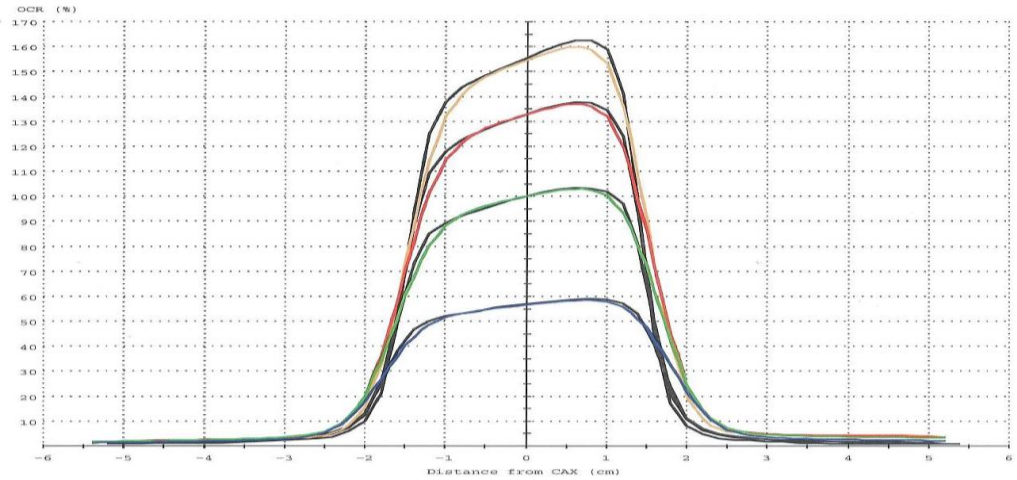

Fig. 4 a: wedge profile for 3 x 3 cm^2 field size

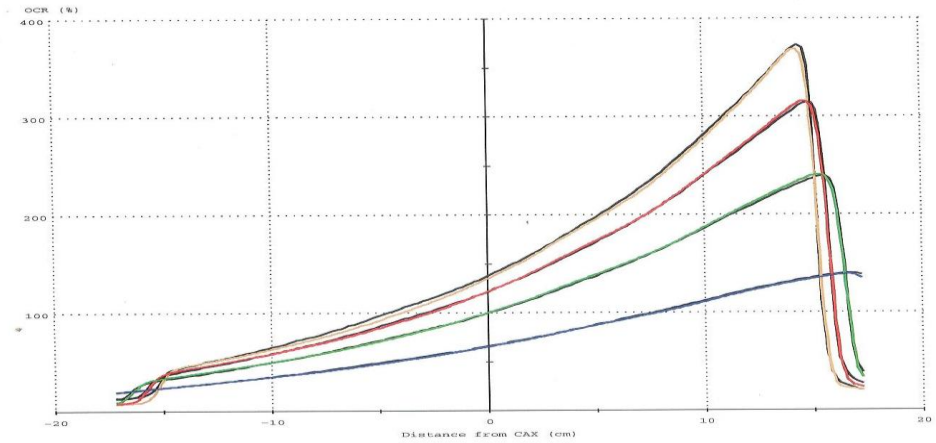

Fig. 4 b: wedge profile for 30 x 30 cm^2 field size

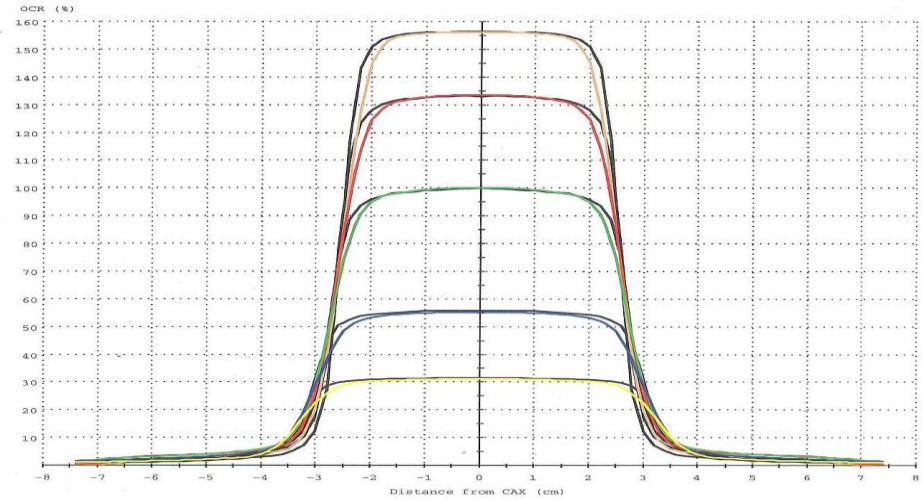

Fig. 5 a: In-plane profile for 5 x 5 cm^2 field size

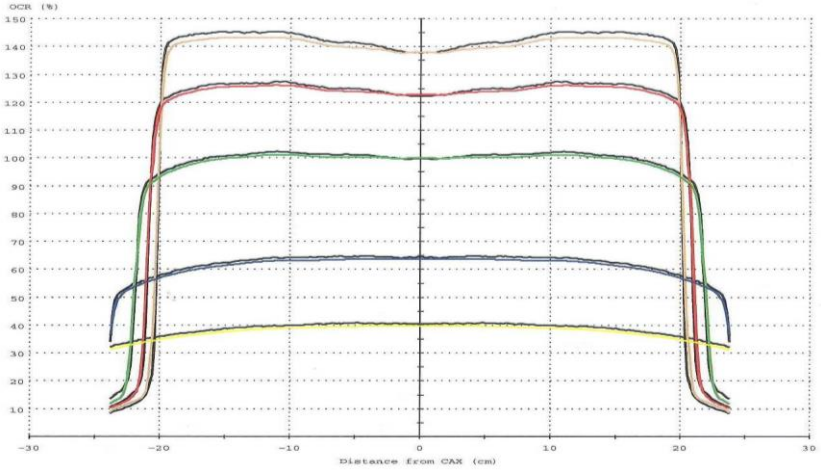

Fig. 5 b: In-plane profile for 40 x 40 cm^2 field size

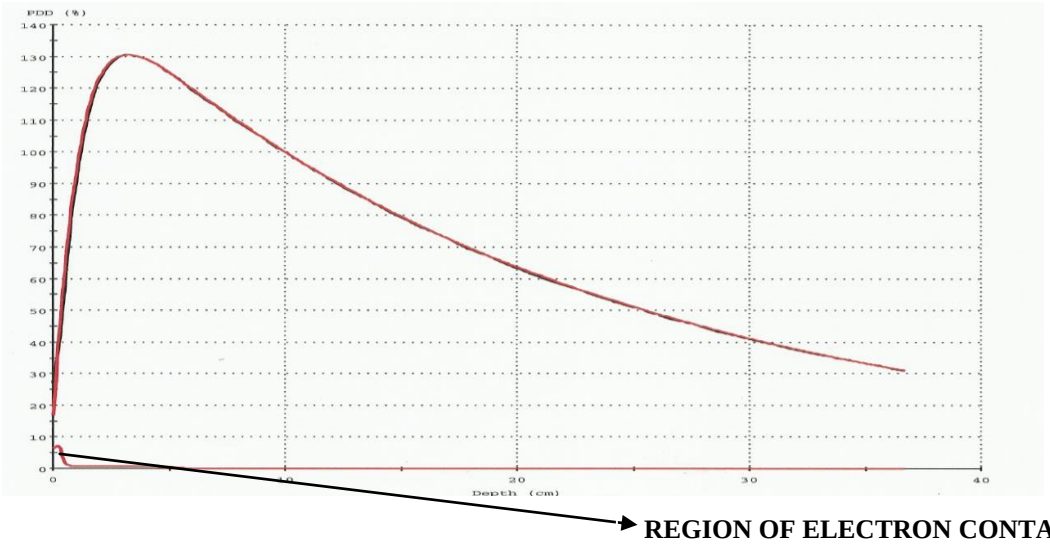

Figure 6 a: Sample of 18MeV normal PDD for 3 x 3 cm^2 field size showing effect of electron contamination.

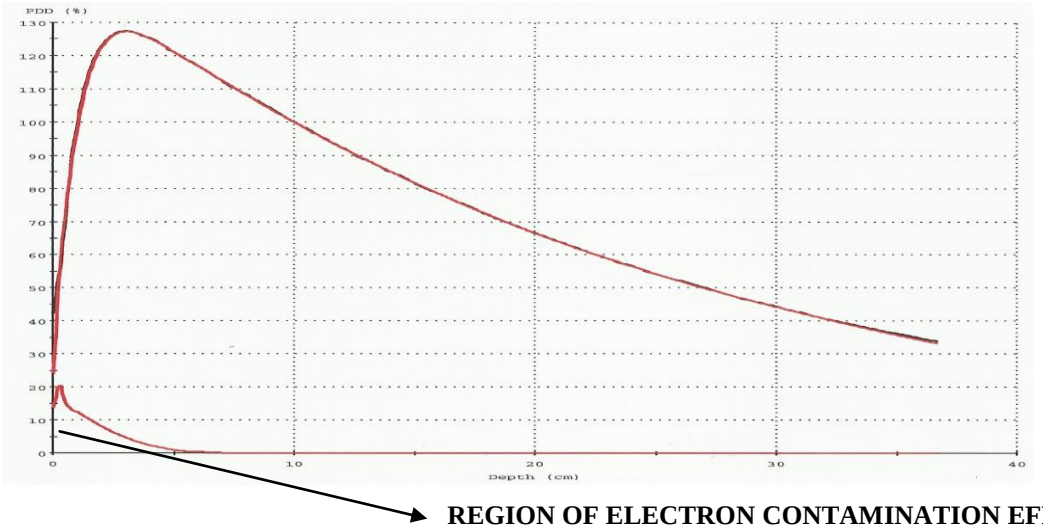

Figure 6 b: Sample of 18MeV normal PDD for 12 x 12 cm^2 field size showing effect of electron contamination.

GARCINIA KOLA EXTRACT AS ANTIFUNGAL THERAPY FOR *CANDIDA* INFECTIONS IN HIV AND NON-HIV PATIENTS - A COMPARATIVE STUDY

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ABSTRACT

Introduction: *Candida*, a normal commensal of the mouth is the cause of candidal infections of the oral cavity in patients with compromised immunity. **Objectives**: To determine the effect of *Garcinia kola* extract on oral *Candida* infection in HIV and non-HIV participants and to compare its efficacy with Chlorhexidine mouthwash. **Materials and methods**: A double blinded clinical trial was carried out in participants with clinically diagnosed *Candida* infection. Consecutive patient presenting with oral candidal infection were recruited until the sample size was achieved. *Garcinia kola* extract and chlorhexidine mouthwash were administered to the participants by the assistant. Data generated was analyzed using the statistical Package for Social Sciences for Windows version 16.0, SPSS Inc., Chicago IL. **Results**: One hundred and twenty participants completed the study, 39(32.5%) males and 81(67.5%) females (m:f = 1:2.1). In non-HIV participants, 27 (96.4%) had the lesion cleared with *Garcinia kola* while 25(80.5%) had it cleared with chlorhexidine. In HIV participants, 27 (81.8%) also had it cleared with *Garcina kola* while 20 (71.4%) had it cleared with chlorhexidine at the end of the 3rd week. There was a statistical significant difference (p=0.01) at the end of the 2nd week in non-HIV participants with 16 (57.1%) having it cleared with *Garcinia kola* and 6 (19.4%) with Chlorhexidine. **Conclusion**: *Garcinia kola* and Chlorhexidine were both effective against candidal infection, but *Garcinia kola* showed a better clinical outcome.

***Keywords**: Candida, Garcinia kola, Chlorhexidine,*

INTRODUCTION

Candida is a normal commensal in the mouth and the oral carriage of the organism is high, with nearly one half of the healthy population harbouring this organism [1] The organism is the cause of common infections of the oral cavity, oesophagus, skin, gastro-intestinal tract, vagina and vascular system of human [1]. *Candida* infection may occur when the host immunological state has been compromised, for instance in patients with leukemia, oral cancer and HIV/AIDS; patients on immunosuppressive drugs following organ transplant; in metabolic diseases e.g. Diabetes mellitus; anaemia, malnutrition, inhalation of steroids in asthmatic patients, chronic use of antibiotics, chemotherapy, denture wearing and gastrointestinal tract diseases (e.g. coelic disease, chron's disease and ulcerative colitis).

The current treatment of *Candida* infection in the oral cavity is through the use of antifungal drugs e.gs Nystatin, Miconazole, fluconazole e.t.c. Although these drugs are currently available in the country, many of them have been associated with side effects which include nausea, vomiting, diarrhea, anorexia, severe allergic reaction e.t.c. All these are known to effect drug compliance. Other mitigating factors include cost and drug resistance. [2, 3, 4]

The use of medicinal plants for the treatment of infections/ diseases is becoming a current trend in developed and developing countries. Thus, using drugs derived from plant in the treatment of

any disease particularly where resistance can occur to the present drug and which will also be useful in the treatment of HIV infected patients should be welcomed.

Garcinia kola belongs to the family of a tropical plant called Guttiferae. It is found in West and Central African sub-regions (Nigeria, Sierra-leone, Ghana, Cameroon and Congo) [5].The plant has the popular acronym 'wonder plant' among the South Western Nigerian people because every part of it has been found to be of medicinal value[6]. It is a medium-sized evergreen tree, mostly about 12m high and sometimes up to 28m in height, and 1-5m width[7]. The plant is cultivated for its edible fruits (used as food and tonic). Studies on the fruits of *G. kola* have resulted in the isolation of the petroleum spirit extract and characterization of kolanone (a novel polyisoprenylated benzophenone) The seeds of *Garcinia kola* contain several known simple flavonoids together with the biflavonoids (GB-1, GB-1a and GB-2, Garcinal and Garcinoic acid) and amentoflavonone (hydroxybiflavononols presence in the ethyl acetate fraction).These account for its antifungal, antibacterial and antiviral properties which have been demonstrated in many studies. As antifungal against Candida infection of the eye by Adefule-Ositelu et al[8], reduction of intraocular pressure and miosis by Adefule-Osditelu et al[9] ; as antibacterial by Adefule-Ositelu[10] and Sote et al[11], as antiviral by Adefule- Ositelu et al[12] , reducing pain and sub-chondral pressure in knee osteo-arthritis by Adegbehingbe[13]. We therefore set to investigate its antifungal activity against oral *Candida* and to observe any significant side effects.
Another treatment option for Oral candidiasis is Chlorhexidine (1:6-di-4-chlorphenyl diguanidohexane). It is a broad spectrum antibacterial agent; bacteriostatic at low concentrations and bacteriocidal at high concentrations[14]. It is has antifungal properties. It is cheaper than the conventional antifungal drug. Hence the preferred choice for comparison with *Garcinia kola.* The study would therefore be a comparative evaluational study.

METHODS

Study Design: It was a double blinded clinical trial which was carried out in participants with clinically diagnosed *Candida* infection.

Sample Selection:
Participants for the study were recruited from the Oral Medicine Clinic of LUTH, Randle General Hospital, APIN clinic, LUTH and General Hospital, Lagos Island. In each of the clinics, every consecutive patient presenting with oral candidiasis were recruited until the sample size was achieved.

Consent:
Written informed consent, were obtained from all the participants before enrollment in the study. Proforma were used to collect the data.

Ethical Clearance:
Approval for the study was obtained from the Health Research and Ethics committee (HREC) of Lagos University Teaching Hospital (LUTH); Randle General Hospital, Surulere and General Hospital, Lagos Island.

Preparation of *G.KOLA* Extract
About 4000g of *G.kola* nuts were bought from Oyingbo market in Lagos, Nigeria. The plant was identified, authenticated and assigned a voucher specimen number Lagos LUH 3688 which was deposited at the University of Lagos herbarium. A concentration of 30% (30g in 100ml) of *G.kola* was obtained by cold extraction process which was as close to ordinarily chewing the nut under hygienic condition. This involved a cold extraction process.

The seed coats on the nuts were scrapped off to expose the white inner parts. These were washed and cut into pieces and blended using Kitchen blender (Binatone). The water extraction mixture obtained was poured into a beaker and allowed to stand on the bench for 15hours[15]. It was filtered and the extract (supernatant) was preserved in the refrigerator for the clinical trial.
The concentration of chlorhexidine used was 0.2%.

Data Collection Techniques
The investigator utilized the service of an assistant in carrying out the study. The investigator/examiner made the diagnosis of oral candidiasis based on EEC-Clearing House diagnostic criteria [7].
Equal number of participants were recruited in the two main groups, one group comprised subjects with HIV and the other group comprised non- HIV subjects. They were all screened for HIV infection. Participants in each group were randomly allocated by a trained assistant to two different treatment groups (*Garcinia kola* extract and Chlorhexidine). This was to ensure that both the participants and the investigator were blinded to the medication each participant was receiving. The assistant thereafter dispensed the different mouthwashes to the participants according to the group they have been randomly assigned to. Each participant was instructed to use the mouthwash 3 times daily and were advised not to use the mouth rinses within 30minutes of using fluoride containing toothpastes (this is to prevent inactivation of chlorhexidine). The mouth rinses were dispensed in identical containers, labeled A and B. The assistant kept a record of the treatment received by each participant and the investigator was not aware of the participants' treatment groups until after the final treatment outcome was determined. The participants were advised to swish the solution around the mouth and leave for 1min before spitting out. The treatments were stopped at the end of the 3rd week. During the period of this study, the examiner did not observe any side effect/s of *G.kola* and CHX. Participants whose candidal infection did not clear at the end of the third week were placed on regular antifungal drugs.

Recall Visit
The participants were seen at one week interval and the clinical state of the oral candidal infection was documented, that is if it had cleared completely, reduced or not. There were 3 recall visits in all.

Clinical Effectiveness
The clinical effectiveness of *Garcinia kola* extract and chlorhexidine was determined using these criteria:
Totally cleared (The lesion completely cleared in the oral cavity).
Partially cleared or reducing (the lesion partially cleared in the oral mucosa).
Not cleared (the lesion remained the same).

RESULTS
A total of 132 participants who were clinically diagnosed with *Candida* infection were enrolled for the study but a total of 120 participants completed the study and were analyzed. The response rate was thus 90.9%.

Sex distribution
Table 1 shows the sex distribution pattern in HIV positive and HIV negative participants. Females were more prevalent in both groups. However the number of females was higher in the HIV positive group (70.5%) compared with the HIV negative group (64.4%) but the difference was not statistically significant ($p = 0.61$).

Pattern of age distribution in the participants

In the 120 participants, the overall age range was 18 – 93 years (mean age 44 ± 16 years. Majority of the subjects were in the fourth and fifth decades of life with a peak incidence in the fourth decade.

A higher no 23(37.7%) of participants in the HIV positive group were in the 30-39years age group while for the HIV negative group, a higher number of the participants 21(35.6%) were in the ≥60 years age group. The mean age of the 61 participants in the HIV positive group was 37.2 ± 11.0 years while the mean age of the 59 participants in HIV negative group was 51.0 ± 17.5years. The age difference between HIV positive and HIV negative groups was statistically significant ($p < 0.005$).

Presence of associated medical conditions in HIV positive and HIV negative participants

Table 3 shows that most of the subjects did not have associated medical conditions. However, a higher proportion of the HIV negative group 14 (23.7%) had associated medical conditions compared to the HIV positive group 2(3.3%). This difference was statistically significant (P=0.003). The medical conditions seen were diabetes 3(5.1%), hypertension 8(13.5%), Systemic lupus erythematosus 1(1.7%), hemorrhoids 1(1.7%), cancer of the palate 1(1.7%).

Even though, there was a statistical significance difference, the clearance of *Candida* by the two mouthwashes was not affected by the medical conditions. The diabetes participants had their blood sugar controlled with glycated heamoglobin of 5.1% (>8%), 6.0% (>8%) and 8.2% (6.2-8.6%). The *Candida* infection seen in the systemic lupus erythematosus subject was due to steroid used during the flares of the condition and that of subject with cancer of the palate was post radiotherapy.

Comparison of the treatment outcomes of *Garcinia kola* with chlorhexidine in HIV positive and HIV negative participants.

HIV positive participants: In week one, the lesion was not totally cleared in the participants using *G.kola* and CHX. In week two, 10 (30.3%) participants in *G.kola* group and 7(25.0%) in CHX had it totally cleared. In week three, 27 (81.8%) participants had it totally cleared in *G.kola* group while 20 (71.4%) had it in CHX

HIV negative participants: In week one, the lesion was not totally cleared in the participants in both groups. In week two, 16 and 6 participants in *G.kola* and CHX group respectively had it totally cleared while in week three, 27 and 25 participants had it totally cleared in *G.kola* and CHX group respectively.

There was no statistical significant difference in signs between the *G.kola* and CHX participants in HIV positive group, however there was a statistically significant difference between the *G.Kola* and CHX group in week 2 in HIV negative participants, $p < 0.05$.

DISCUSSION

Females were predominant in both HIV and non-HIV groups as seen in Table 1. Similar observation has been reported in the study by Kantheti LP et al [16] to determine the prevalence of *Candida* colonization. The study was carried out among HIV negative subjects (group 1); HIV positive but naive to highly active anti-retroviral therapy (HAART) (group II) and HIV positive with less than 250 CD4 count (group III).

It was observed that there was a slight female preponderance in group II (Female: Male= 24: 22). Although in group I and group III, males were more prevalent (Female: Male= 6: 16 and 18:20 respectively) and this was different from our observation[16].
In the HIV positive subjects, candidal infection was mostly seen in the age 30-39years with prevalence of 23(37.7%). This is in agreement with studies by Vander waal et al[17], Bodhade AS et al [18]. It can be said that this age group are sexually active compared to the elderly. Sexual transmission is one of the highest means of transmission of HIV/AIDS in this environment [18]. However in another study, 40-49 years has been reported to have the highest prevalence [19]. Age ≥60years had the least infection. An average HIV carrier is a young and active person usually in the third or fourth decade of life[18].

The presence of an underlying medical condition did not affect the treatment outcome of candidal infection in this study because the medical conditions were controlled. Samaranayake demonstrated the presence of associated medical conditions with *Candida* and state that the growth of *Candida* is enhanced by the presence of an underlying medical condition [20].

The overall success rate of lesion totally cleared by the end of the third week by using 30% of *Garcinia kola* was 88.5% and 76.3% using 0.2% chlorhexidine. In HIV positive subjects, the success rate of *Garcinia kola* was 81.8% and 71.4% for chlorhexidine. In HIV negative subjects, the success rate of *Garcinia kola* was 96.4% while Chorhexidine was 80.6%. A statistical significant difference in the treatment outcome between *Garcinia kola* (57.1%) and chlorhexidine (19.4%) was observed in week two (p=0.01)

The antifungal effect of chlorhexidine has been demonstrated in several clinical trials. It has been used successfully in a regimen for the treatment of oral candidiasis in otherwise healthy individuals [21, 22, 23]. A study done by Barsch et al demonstrated its efficacy on HIV positive subjects. The efficacy of Chorhexidine has also been demonstrated in the elderly patient with oral candidiasis by Persson et al [24]. They observed that there was no statistical difference between Chlorhexidine and Nystatin; further proving its efficacy. Furthermore, Ellepola showed its importance as an adjunct in the treatment of *candidal* infection with conventional antifungal drug which has a short duration of action (chlorhexidine has a long duration of action) supporting its use as an alternate oral antifungal therapy[25]. Barkvoll et al emphasized using Nystatin 30minutes before Chlorhexidine due to the low soluble complex salt formed, Chlorhexidine - Nystatin complex that is less effective [26].
Although, its efficacy has been demonstrated, Chlorhexidine exhibits some short comings which are: teeth and mucosal staining, mucosal desquamation, parotid gland enlargement on vigorous rinsing. Also, it is not readily available in an average Nigerian pharmacy shop. The non-availability, its side effects undermines the choice of chlorhexidine when considering the advantages of *Garcinia kola* which are affordability, availability, non-toxicity and no side effects reported in the literature and experienced so far make *Garcinia kola* superior and preferable to chlorhexidine solution.

From the study, the success rate of *Garcinia kola* was demonstrated in both HIV positive and HIV negative participants, this is a major discovery. This study supports the use of *Garcinia kola* as against Chlorhexidine as antifungal therapy coupled with its advantages over chlorhexidine. However in HIV participants, could there have been better results if they complied very well? Could a higher concentration have better effect?
Interest in plant extracts exhibiting antimicrobial and pharmacological applications is on the increase recently. Nigeria climate favours great array of plant species, many of which have

varied medicinal and antimicrobial potentials. Medicinal uses of plants range from the roots, barks, stems, leaves and seeds of extracts.

Garcinia kola has been shown to be anti-*Candida* amongst many other plants like Rubiaceae, Abibacceae, Periplocaceae, Zingiberaceae [27]. Kagbo cited a work by Bohn, where antifungal property of *Garcinia kola* was demonstrated[28]. A research by Adefule Ositelu demonstrated that *Garcinia kola* was effective by comparing it with standard anti-microbial agents on common eye fungal and bacterial micro-organisms. These organisms were *Candida albicans, Bacillus subtilis, Stapylococcus aureus, Streptococcus pneumonia, Enterococcus faecalis, Escherichia coli,* and *Klebsiella pyocyannae* amongst others [8]. Indeed, medicinal plants are important source of new chemical substances with potential therapeutic benefits [29]. This has also been successfully demonstrated in this study on *Garcinia kola.*

In conclusion, can we answer this question, why the use of *Garcinia kola*? It is a plant with medicinal value. It is cheap, edible, readily available, non-toxic and in this study, no observable side effects was noticed with *Garcinia kola,* as also reported in other studies [13, 35].

Further Researches: There is the need for further researches on this medicinal plant and its effect on oral candidal infections. The subjects whose candidal infection did not clear at the end of the third week may be due to the other species of *Candida* for example *Candida glabrata*, *Candida krusei* and *Candida topicalis* which are more difficult to treat. A biochemical and genetic analysis may be done to identify these species in another study when further determining the efficacy of *Garcinia kola*. It may be modified either by increasing the dosage or frequency. This will be more encompassing and contribute to the body of knowledge. Further studies are needed and studies are already on-going on HIV positive patients and *Garcinia kola*.

CONCLUSION

Garcinia kola and chlorhexidine were both effective against candidal infection, but there was a statistical significant difference between the clinical success rate of *Garcinia kola* and chlorhexidine in HIV negative subjects at the end of the second week, with *Garcinia kola* showing a better clinical outcome. There was no statistical significant difference between *Garcinia kola* and chlorhexidine in the HIV positive group.

Subjects did not complain of any side-effects of *Garcinia kola* and none was observed by the investigator during review.

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Table 1: Sex distributions of participants

Sex	HIV positive		HIV negative	
	Frequency	**Percent**	**Frequency**	**Percent**
Male	18	29.5	21	35.6
Female	43	70.5	38	64.4
Total	**61**	**100**	**59**	**100**

$\chi^2 = 0.27$, df = 1, p = 0.61

Table 2: Age distribution of participants

Age (year)	HIV positive (n = 61)		HIV negative (n = 59)		Total (n = 120)		Fisher exact p
	Frequency	**Percent**	**Frequency**	**Percent**	**Frequency**	**Percent**	
< 20	3	4.9	3	5.1	6	5.0	0.0001*
20 – 29	11	18.0	3	5.1	14	11.7	
30 – 39	23	37.7	11	18.64	34	28.3	
40 – 49	15	24.6	10	16.94	25	20.8	
50 – 59	7	11.5	11	18.64	18	15.0	
≥ 60	2	3.3	21	35.59	23	19.2	
Total	**61**	**100**	**59**	**100**			
Mean age	37.2 ± 11.0		51.0 ± 17.5		44.0 ± 16.0		
Median	35		52		42		

Student's t statistics = 5.19, p < 0.001*

*Significant

Table 3: Presence of associated medical conditions in HIV positive and negative subjects

Presence of other medical conditions	HIV positive		HIV negative	
	Frequency	**Percent**	**Frequency**	**Percent**
Present	2	3.3	14	23.7
Absent	59	96.7	45	76.3
Total	**61**	**100**	**59**	**100**

$\chi^2 = 9.16$, df = 1, p = 0.003*

*Significant

Table 4: Treatment outcome in HIV positive participants

Time post treatment	Outcome of symptom	Frequency (%)		χ^2	p-value	Fisher exact p
		Garcinia kola	Chlorhexidine			
		n = 33	n = 28			
Week 1	None	0 (0)	0 (0)			1.00
	Totally cleared	0 (0)	0 (0)			
	Partially cleared	32 (97.0)	28 (100)			
	Not cleared	1 (3.0)	0 (0)			
	Total	**33 (100)**	**28 (100)**			
Week 2	None	0 (0)	0 (0)			
	Totally cleared	10 (30.3)	7 (25.0)	0.03	0.86	
	Partially cleared	23 (69.7)	21 (75.0)			
	Total	**33 (100)**	**28 (100)**			
Week 3	None	0 (0)	0 (0)			
	Totally cleared	27 (81.8)	20 (71.4)	0.43	0.51	
	Partially cleared	6 (18.2)	8 (28.6)			
	Total	**33 (100)**	**28 (100)**			

*Significant

Table 5: Treatment outcome in HIV negative group

Time post treatment	Outcome of symptom	Frequency (%)		χ^2	p-value	Fisher exact p
		Garcinia kola	Chlorhexidine			
		n = 28	n = 31			
Week 1	None	0 (0)	0 (0)			1.00
	Totally cleared	1 (3.6)	1 (3.2)			
	Partially cleared	27 (96.4)	30 (96.8)			
	Not cleared	0 (0)	0 (0)			
	Total	**28 (100)**	**31 (100)**			
Week 2	None	0 (0)	0 (0)			
	Totally cleared	16 (57.1)	6 (19.4)	7.44	0.01*	
	Partially cleared	12 (42.9)	25 (80.6)			
	Total	**28 (100)**	**31 (100)**			
Week 3	None	0 (0)	0 (0)			0.11
	Totally cleared	27 (96.4)	25 (80.6)			
	Partially cleared	1 (3.6)	6 (19.4)			
	Total	**28 (100)**	**31 (100)**			

*Significant

OPEN ACCESS PUBLISHING: A REVIEW OF PUBLICATIONS EMANATING FROM A MEDICAL COLLEGE IN NIGERIA

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ABSTRACT
Background: Open Access (OA) publishing has gained tremendous acceptance in academic publishing over the last decade. Open Access denotes a policy of unrestricted and free access to academic publications on the Internet. It is the practice of providing unrestricted access via the Internet to peer-reviewed scholarly journal articles. **Open access** (OA) to the research literature has the potential to accelerate recognition and dissemination of research findings, but its actual effects are controversial. **Aim:** This paper reviews the number and trend of Open Access Publishing of research papers emanating from College of Medicine University of Lagos, Nigeria. **Methodology:** A computerized literature search of PubMed for all published articles emanating from CMUL between 1976 and 2013 was conducted. Search phrase used was "College of Medicine University of Lagos". The search was conducted on the 30th March 2013. All articles tagged "Free article" or "Free PubMed article" were selected. **Results:** A total of 1255 articles appeared in PubMed between 1976 and 2013 (37 years). At the first level of screening, a total of 162 articles were identified as "Open Access". Second level of screening to eliminate articles not emanating from CMUL identified 124 articles. Only 15 Open Access articles were published between 1976 and 2000 (24 years), 11 articles appeared as "Open Access" journals between 2001 and 2005 (5 years), 44 between 2006 and 2010 (5 years) and 54 articles were published between 2011 and 2013 (less than 3 years). Twenty-four of these articles were published in Nigerian Open Access Journals, and the rest (100) in foreign journals. **Conclusions:** Open Access publishing is becoming popular among researchers at the CMUL. This trend has been observed Worldwide. Nigerian researchers are advised that while going along with the worldwide trend, they should however, be aware of Predatory Open Access Journals and Publishers. Criteria for determining Predatory Open Access Publishers can be accessed via: www.scholarlyoa.com/publishers.

Keywords: *Open access; publishing; medical college;predatory*

INTRODUCTION

Open access (OA) is the practice of providing unrestricted access via the Internet to peer-reviewed scholarly journal articles.[1-3] Open Access (OA) publishing has gained tremendous acceptance in academic publishing over the last decade.[1-3] Open access (OA) to the research literature has the potential to accelerate recognition and dissemination of research findings, but its actual effects are controversial.[4] The following categories of Open Access are recognized: Gold Open Access, Green Open Access (Self Archiving), and Hybrid Open Access.[2] Gold OA means scientific journals are openly accessible, and the Green OA denotes that publications are self-archived in repositories. Hybrid OA journals are subscription journals that provide gold open access only for those individual articles for which their authors (or their author's institution or funder) pay an open access publishing fee.[2-4]

With the growing use of the internet, new publishing models are emerging, which are committed to providing free access to the full text of research articles.[3] Many society-owned journals now offer their archives online free of charge, while retaining subscription-only access to newer

material. The challenge now is to make access free from the moment of publication in a way that has long term sustainability.[3]

Many research funding agencies like National Institute of Health (NIH) and Welcome Trust have endorsed open access policy. Many governments, especially in the developed worlds are also openly endorsing OA policy. Many subscription based publishers (Elsevier, Springer) now give authors an option of "Open Access" for their articles. Once the authors agree and pay the fee, the full text of their research work can be accessed by other researcher without subscription. Studies have also shown that OA articles are more likely to be used and cited than one behind subscription barriers.[4] However, it must be recognized that ease accessibility to the internet has created many "junks" in Open Access publishing. Researchers must be wary of such journals otherwise called "Predatory OA journals" as described by "Open Access Scholarly Publishers Association" (http://oaspa.org/), and the list can be found here: (http://scholarlyoa.com/)

In Nigeria, anecdotal evidence suggests that many peer-reviewed journals as well as scholars are now embracing Open Access publication. It is important to explore the attitude of Nigerian researchers to the "Open Access" publication and compare with trends in other part of the world. Therefore, this paper reviews the trend of Open Access Publishing of research papers emanating from College of Medicine University of Lagos (CMUL) between 1976 and 2013.

METHODS

A computerized literature search of PubMed for all published articles emanating from the College of Medicine University of Lagos (CMUL) between 1976 and 2013 was conducted. Search phrase used was "College of Medicine University of Lagos". The search was conducted on the 30th March 2013. All articles tagged "Free article" or "Free PubMed article" were selected as Open Access articles. Two levels of screening for the articles were employed during the search. The search phrase "College of Medicine University of Lagos" was used for the first level. At the second level of screening, all the articles that appeared at the first level of screening were manually searched and only those that strictly have College of Medicine University of Lagos address were selected for analysis.

RESULTS

A total of 1255 articles appeared in PubMed between 1976 and 2013 (37 years). At the first level of screening, a total of 162 articles were identified as "Open Access". Second level of screening to eliminate articles not emanating from CMUL identified 124 articles. A total of 124 articles from CMUL appeared in Open Access Journals. Only 15 Open Access articles were published between 1976 and 2000 (24 years), 11 articles appeared as "Open Access" journals between 2001 and 2005 (5 years), 44 between 2006 and 2010 (5 years) and 54 articles were published between 2011 and 2013 (less than 3 years). Figure 1 shows trend of Open Access Publication from the College of Medicine University of Lagos. The trend reveals a steady rise in OA articles particularly between 2001 and 2013. Twenty-four of these articles were published in Nigerian Open Access Journals (Table 1), and the rest (100) in foreign journals.

DISCUSSION

There is currently an explosion of interest in the academic and publishing communities about the promise—and possible perils—of open-access scholarship and publishing.[1] According to Chatteriee et al[4] the debate on open access to scientific literature that has been raging in scholarly circles for quite some time now and has been fueled further by the recent developments in the realm of the open access movement. According to Eysencbach,[5] there are two parallel ''roads'' towards OA: OA journals and self-archiving. OA journals make published articles immediately freely available on their Web site, a model mostly funded by charges paid by the author (usually through a research grant). The alternative for a researcher is ''self-archiving'' (i.e., to publish in

a traditional journal, where only subscribers have immediate access, but to make the article available on their personal and/or institutional Web sites [including so-called repositories or archives]), which is a practice allowed by many scholarly journals.[5]

The quality of articles appearing in OA journals and subscription-based journals has also been a source of debate.[1,3,5] Some scholars have also queried the review process of OA journals believing the process is not rigorous enough.[6] However, other authors have reported no difference on the basis of impact factors between OA journals and subscription-based journals.[6] Sabharwal et al[7] also suggests equivalent importance and quality of articles between OA and subscription based orthopaedic journals based on bibliometric data and the volume of level I evidence produced.

In a longitudinal bibliometric analysis of a cohort of OA and non-OA articles published between June and December 2004 in PLOS Biology, Eysenbach[5] reported that average number of citations of OA articles was higher compared to non-OA articles. According to Eysenbach[5] OA articles compared to non-OA articles remained twice as likely to be cited in the first 4–10 months and 10–16 months after publication. He found strong evidence that, even in a journal that is widely available in research libraries, OA articles are more immediately recognized and cited by peers than non-OA articles published in the same journal.[5] He concluded that OA is likely to benefit science by accelerating dissemination and uptake of research findings.[5]

An increasing trend in Open Access Publishing was observed in the publications emanating from College of Medicine University of Lagos, Nigeria. This trend is commendable and in line with what goes on around the academic World. It is also commendable that all the five open access journals emanating from Nigeria are either institutional- or society-based journals. This implies that our institutions and medical societies are already imbibing open access policy.

It must be stated that ease accessibility to the internet has created many "junks" in Open Access publishing. There are thousands of journals and publishers. However, researchers must be able to "separate the wheat from the chaff" OA journals when submitting their publications to open access journals. Researchers must look out for "predatory journals and publishers" when submitting their papers for publications. A search through the following websites can be of help: (http://oaspa.org/), and (http://scholarlyoa.com/). Criteria for determining Predatory Open Access Publishers can be accessed via: www.scholarlyoa.com/publishers.

Although there are no foolproof methods to find out fake journals (predatory journals/publishers) in open access platform, but scholars according to Sau[8] can take certain measures to avoid these kind of counterfeit journals. First, they should check scope of the journal as most of these predatory journals publish articles from different fields of science[9] within a short period of time[9,10], which is usually not possible for a normal regular journal.[8] The code of conduct of Open Access Scholarly Publishers Association (OASPA) makes it mandatory that an OA journal should have editorial boards or other governing bodies consisting of recognized specialists from the field(s) that constitute the scope of the journal. OASPA also suggested that there should be some form of peer review and it should be clearly mentioned on the journal or publisher's website. The details of the Code of Conduct for Open Access Publishing can be found the OASPA website: (http://oaspa.org/).

The fact that the method used in this study only explored articles that appeared in "PubMed search engine" is considered a limitation of the study. In addition, the scope is focused only on an institution (CMUL). However, it is our opinion that the present study forms a basis for understanding the trend of open access publishing in medical research in Nigeria.

CONCLUSION

Open Access publishing is becoming popular among researchers at the CMUL. This trend has been observed Worldwide. Nigerian researchers are advised that while going along with the

worldwide trend, they should however, be aware of Predatory Open Access Journals and Publishers. With this new trend observed among researchers, our libraries should also be equipped to pursue Open Access publishing alternatives in addition to subscription-based journals.

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PATTERN OF ORDERING AND USAGE OF HOMOLOGOUS BLOOD TRANSFUSION FOR MAJOR ELECTIVE MAXILLOFACIAL SURGERY AT THE LAGOS UNIVERSITY TEACHING HOSPITAL

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ABSTRACT
Background: Justification for the requests for homologous blood that accompany major elective maxillofacial surgical procedures is difficult to establish in most cases. This attitude of ordering for cross-matched blood is understandable in today's legal climate, but has led to serious problems in terms of laboratory inefficiency which can no longer be ignored. **Objective**: To evaluate the pattern of ordering and use of homologous blood for major elective maxillofacial surgeries at the Lagos University Teaching Hospital (LUTH), Idi-Araba Lagos. **Patients and Methods**: Sixty-three consecutive subjects who required major elective maxillofacial surgery under general anaesthesia, and who met the inclusion criteria were included in the study. Data collected included age, sex, weight, and height of subjects, type of surgery done, preoperative and intraoperative haemoglobin concentration, blood units cross-matched and units transfused intraoperatively. Each subject was made to donate through a representative donor, at least one unit of homologous blood prior to surgery. **Results**: There was a male predominance (57.1%) among subjects, with male to female ratio of 1.3: 1. Mean age of subjects was 33.9 ± 13.5 years. O+ was the most predominant blood group (62%). Tumours (58.8%), were the most common indication for surgery. Majority of subjects (95.2%), had a preoperative haemoglobin concentration of ≥ 10g/dl. Haemoglobin concentration at the point of transfusion was < 10g/dl for 58.8% of transfused subjects. The overall cross-match to transfusion ratio was 3.35, overall probability of transfusion was 26.9%, while the overall transfusion index was 0.6. Only oncological surgical procedures showed an efficient blood usage in all the 3 indices. **Conclusion**: This study demonstrated that only onclogical surgical procedures have an indication for cross-matching of blood for surgery, however there is a need to determine the maximum surgical blood ordering schedule for these procedures. There is therefore the need to change the blood ordering pattern, and minimize over-ordering of blood for major elective maxillofacial surgery.

***Keywords**: Homologous blood transfusion, Elective oral & maxillofacial surgery, Transfusion indices*

INTRODUCTION

Major elective maxillofacial surgical procedures involve operations for tumours, trauma, or congenital malformations in the head and neck region.[1-3] One of the major complications these operations is the potential for excessive blood loss being, especially in large tumours at advanced stages that often require extensive excision.[3-5] In such cases, transfusion of blood units may become necessary.[6,7]

All parties involved in maxillofacial surgery, the maxillofacial surgeon, anaesthesiologist, and the patient are interested in the expected operating time and anticipated need for blood transfusion.[8]

The indications and trigger for blood transfusion have been redefined in the last decade to ensure that blood and blood products are considered and treated as medications in their own merit.[15] Although a haemoglobin concentration of 10 g/dl is considered a safe one at which to allow

operation under general anaesthesia for a normal cardio-respiratory system with intact compensatory mechanisms,[7-9] nowadays, operations can be performed successfully at haemoglobin concentration of 8 g/dl.[10,11]

Previous reports have indicated that it is possible to achieve considerable cost savings by changing the blood ordering practices of physicians without compromising the quality of patient care.[12] The over-ordering of cross-matched blood to cover operation can result in blood shortages, is costly and cannot be free of risk.[3] By realigning cross-matching orders with actual expected needs, substantial savings can be realized in terms of personnel time, reagents, and outdating of units of blood.[13] One of the standard methods used as a quantification of this problem and so determine the efficiency of blood ordering, is to determine the ratio of units cross-matched to units transfused, the cross-match/transfusion ratio (C:T ratio). [14,15] For a hospital with a full range of clinical services the C:T ratio should be about 2.5:1.[15] A considerably higher ratio indicates that there have been areas where excessive ordering has taken place.

It has been observed that homologous blood is often requested for every patient undergoing major elective surgeries whether depending on the claim of the surgeon or the anaesthesiologist for needing it.[7] Since a large number of units of blood is ordered for and a significant number is administered to patients undergoing major elective maxillofacial surgical procedure, there is a need to study the current pattern of ordering and use of homologous blood in order to assess how much cross-matched blood is wasted.[6]

The present study therefore seeks to determine the efficiency of blood ordering practice, and to evaluate the pattern of use of homologous blood in patients undergoing major elective maxillofacial surgical procedures at the Department of Oral/Maxillofacial Surgery, Lagos University Teaching Hospital, Idi-Araba Lagos.

METHODS

Consecutive subjects who required major elective maxillofacial surgery under general anaesthesia, and who met the inclusion criteria for this study were included in the study between December 2011 and December 2012. The weight and height of subjects were obtained. Five milliliters of venous blood was obtained from subjects 24 hours before surgery, and analysed for haemoglobin concentration at the laboratory. Baseline haemodynamic variables were obtained for each subject, by the anaesthetist, upon arrival at the theatre red line. All surgeries were performed under strict aseptic technique, under general anaesthesia. Haemodynamic variables were measured, and five milliliters of venous blood, obtained at the point of transfusion from all subjects that were to be transfused. The blood sample was then sent to the laboratory immediately for analysis of haemoglobin concentration, while transfusion was ongoing. Intraoperative blood loss was estimated at the end of the procedure by measuring the surgical blood soaked gauze, and the blood in the suction bottle, with subtractions made for dilution fluid, before adding them together.

RESULTS

There was a male predominance (57.1%) among subjects, with male to female ratio of 1.3 : 1. Mean age of subjects was 33.9 ± 13.5 years. O+ was the most predominant blood group (62%). Tumours (58.8%), were the most common indication for surgery, followed by maxillofacial trauma (19.0%). (Table A) One hundred and twenty four units of homologous blood (range = 1 – 4 units) were cross-matched for surgery as ordered by the anaesthesiologists in conjunction with the surgeons.

Preoperative haemoglobin concentration values for all subjects ranged between of 9.2 - 16.6mg/dl, (mean = 12.3 ± 1.6 mg/dl). Most of them (95.2%) had values of ≥10 mg/dl. There

was no statistically significant association between the means of preoperative haemoglobin concentration and the cross matched blood ordered for subjects (P = 0.645). (Table C) Majority of transfused subjects (58.8%), had haemoglobin concentration of < 10g/dl at the point of transfusion. There was a statistically significant inverse relationship (P = 0.007, Pearson Correlation = - 0.63) between haemoglobin concentration at the point of transfusion and the number of units of blood transfused.

Overall mean estimated blood loss was 867.3 ± 736.4ml. Oncological surgical procedures had the highest number of units of cross-matched and transfused blood. This was followed by salivary gland tumour excision with 24 units of blood cross-matched, and only 3 units transfused. (Table D)
Oncological surgical procedures and orthognathic surgical procedures showed significant blood utilization by all the 3 transfusion indices. The overall crossmatch-to-transfusion ratio (C:T ratio), transfusion probability (PoT), and transfusion index(TI) were 3.35, 26.9, and 0.6 respectively. The overall percentage blood utilization was 28.9%. (Table D) No mortality or transfusion reaction of subjects was recorded during the study.

DISCUSSION

Pre-operative over-ordering of blood for elective surgery has been documented since the findings of Friedman et al.[16] was published in 1976. Several other studies,[12-20,] have also reported over-ordering of blood by the surgeons despite the difficulty in mobilizing an equal number of blood donors in most countries.[20] To prevent over-ordering, protocol for elective surgical procedures in some institutions mandates that the surgeon do not make any blood order, or instead may order preoperative type and screen testing, or request only a preparation of 1 unit of packed red cells before the operation.[14-19]
A widely accepted transfusion protocol which has been reported to significantly reduce blood ordering and hence transfusion rate, requires that blood be cross-matched only for patients with a preoperative Hb level < 11g/dl.[13] Despite this, 95.2% (60) of subjects who had Hb ≥ 10g/dl in this study, had 120 units of blood ordered for them (an average of 2 units per subject). This may probably be due to the expected extent of surgery and blood loss. The fact that every patient undergoing a major elective maxillofacial surgery was made to donate at least one unit of blood, irrespective of their preoperative haemoglobin concentrations (95.2% of which had ≥ 10g/dl, mean 12.3 ± 1.6g/dl.) suggests that preoperative haemoglobin concentration was not a strong determinant of the number of units of blood ordered for subjects in this study.
Transfusion guidelines issued by some organizations[11-13] suggested haemoglobin level of 7-8 g/dl as the threshold for transfusion in patients who are not critically ill.[17] Though the correct strategy for transfusion of patients with haemoglobin concentrations between 7 and 10 g/dl is less clear,[15] the need for homologous transfusion in between this range is further defined by clinical indicators.[16] However guidelines and consensus statements over the last decade have consistently expressed the transfusion threshold as a range, usually between 7 and 10 g/dl haemoglobin.[17,18]

The cross-match to transfusion ratio (C:T ratio), is the number of units of blood ordered and cross-matched for each patient, divided by the number of units transfused or used. [42] It reflects the efficiency of blood ordering and usage, and indicates the frequency of use of blood preparations in relation to the amount of blood that has been cross-matched or transfused.[5] According to some studies[1,6,8,13] this ratio should be 1.0 but a ratio of ≤ 2.5 was suggested to be indicative of efficient blood usage. It has been recommended that for procedures with a high likelihood of blood transfusion, the number of units cross-matched should be twice the median requirement for that surgical procedure (cross-match-to-transfusion [C:T] ratio of 2:1).[20] A C:T

ratio of more than 2.5 is said to indicate over-ordering of cross-matched blood, in that 2.5 times more blood is ordered than used, or that < 40% of cross-matched units are transfused.[12] The overall cross-match-to-transfusion ratio (C:T ratio) in this study was 3.35. This indicates an overall over-ordering of cross-matched blood in that 3.35 times more blood was ordered than used, or that < 30% of cross-matched units are transfused. In this study at least one unit of blood was ordered for each procedure in the study, despite the fact that 95.2% of subjects had a preoperative haemoglobin concentration of ≥ 10 g/dl, even for known low-volume blood loss procedures like cleft surgery. Also the anaesthesiologist, depending on how comfortable he is with the judgement of the surgeon on the expected blood loss for a particular procedure, often requested for multiple units of blood for some cases. Several studies [8,9,19] have reported over-ordering as indicated by a C:T ratio that range from 17.6–64.1:1.

The transfusion index (TI), the number of units transfused divided by the number of patients cross-matched, is an index of the average number of units used per patient cross-matched.[1,19,21] A value of 0.5 or more is indicative of the need for a policy of blood grouping, screening for atypical antibodies and saving the serum for future cross-matching if required.[13,14] The overall transfusion index(TI) was 0.6. The number of units of blood transfused in this study is about one half the number of subjects cross-matched. Though the C:T ratio from this study indicates an over-ordering of blood, and that routine cross-matching of blood for all major elective maxillofacial surgical procedures may not be justified, both the PoT and the TI suggest that some major elective maxillofacial surgical procedures may require transfusion for which blood grouping, screening for atypical antibodies and saving the serum for future cross-matching will suffice. The need to find out which major elective maxillofacial surgical procedures will benefit from this policy could be the object of further study.

Oncological surgical procedures and orthognathic surgical procedures showed significant blood utilization by all the 3 transfusion indices. Oncological surgical procedures based on its significant utilization (48.5%), seems to be more likely to need to be given transfusion. This may be due to the fact that it had the highest mean blood loss (1320 ± 718.3 ml) which is an important predictor of transfusion. Salivary gland tumour excision showed insignificant blood utilization by all the 3 transfusion indices. The procedures done under this group of surgery ranged from those that were associated with minimal blood loss such as excision of ranula, to ones that may involve significant blood loss such as parotidectomy, all of which had blood cross-matched for them. In surgeries with insignificant blood usage such as this, only grouping of patients can be done with cross-matching avoided, but with an assurance of availability of blood in the event an emergency situation.[1] The other types of surgery in this study, maxillofacial trauma procedures, cleft surgery, microvascular reconstruction, and other single case procedures that do not fall into the major surgical groupings, did not have any transfusion.

It is therefore necessary to streamline blood ordering and transfusion practices in our environment to minimize over ordering such that, blood will be made available in the operation theatre only in surgeries in which all three indices show significant blood usage. For surgical procedures where the 3 indices showed insignificant blood utilization, only blood grouping should suffice with the serum saved for emergency cross-matching should the need arise.

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Table 1. Indications for surgery in 63 subjects undergoing major elective maxillofacial surgery in LUTH

Diagnosis	N	%
Tumours		
Odontogenic Tumours	17	27
Salivary Gland Tumours	13	20.6
Connective Tissue Tumours	5	7.9
Epithelial Tissue Tumours	2	3.2
Fibrosseous Lesion	2	3.2
Maxillofacial Trauma	12	19.0
Cleft Lip and Palate	7	11.1
TMJ Disorders	3	4.8
OTHERS	2	3.2
Total	**63**	**100.0**

Table 2. Distribution of the means of haemoglobin concentration at the point of blood transfusion (intraoperative) and number of units of blood transfused in 17 subjects that were transfused during major elective maxillofacial surgery in LUTH

Intraoperative Haemoglobin Concentration(g/dl)					Blood Transfused	
N	%		Mean ± SD	Range	n	%
Hb <10	10	58.8	7.9 ±1.01	6.3 - 9.5	26	70.3
Hb ≥10	7	41.2	11.2 ± 0.7	10.2 - 12.3	11	29.7
Total	**17**	**100.0**	**9.22 ± 1.89**	**6.3 - 12.3**	**37**	**100.0**

P = 0.007
Pearson Correlation = - 0.629
N = Number of subjects
n = Number of units of blood transfused

Table 3. Distribution of blood cross-matched and transfused in 63 subjects undergoing major elective maxillofacial surgery in LUTH

Type of surgery	Crossmatched		Transfused	
	Subjects	Units	Subjects	Units
Oncological Surgical Procedures	24	64	14	31
Salivary Gland Tumour Excision	13	24	1	3
Orthognathic Surgical Procedures	2	5	2	3
Maxillofacial Trauma Procedures	12	15	0	0
Cleft surgery	7	8	0	0
Microvascular Reconstruction	2	5	0	0
Others	3	3	0	0
Total	**63**	**124**	**17**	**37**

Table 4. Blood transfusion indices

Type of surgery	CT %	PoT	TI %	Utilization
Oncological Surgical Procedures	2.06	58.3	1.3	48.4
Salivary Gland Tumour Excision	8	7.7	0.23	12.5
Orthognathic Surgical Procedures	1.67	100.0	1.5	60.0
Maxillofacial Trauma Procedures	∞	∞	∞	∞
Cleft surgery	∞	∞	∞	∞
Microvascular Reconstruction	∞	∞	∞	∞
Others	∞	∞	∞	∞
Total	**3.35**	**26.9**	**0.6**	**29.8**

C:T = Cross-match to transfusion ratio (A ratio of 2.5 or less is considered as significant for blood usage)
PoT = Transfusion probability (A value of 30% or more is considered indicative of significant blood usage)
TI = Transfusion index (A value of 0.5 or more is considered indicative of a need for blood grouping, screening for atypical antibodies and saving the serum for future cross-matching if required).
∞ = Value cannot be mathematically defined.

DENTAL AESTHETICS AND THE ORAL HEALTH-RELATED QUALITY OF LIFE OF STUDENTS OF THE UNIVERSITY OF LAGOS

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ABSTRACT

Background: Facial aesthetics affect how people are perceived by society and how they perceive themselves. An unpleasant dental appearance can stigmatize a person, hinder professional achievement, encourage negative stereotypes and have a negative effect on self-esteem. Oral health-Related Quality of Life (OHRQoL) corresponds to the impact of oral health or disease on an individual's daily functioning, wellbeing or overall quality of life. **Objective**: The aim of this study was to determine the relationship between the dental aesthetics of undergraduate students of the University of Lagos and their Oral health-related quality of life. **Methodology**: This was a cross-sectional descriptive study carried out among 420 undergraduate students of the University of Lagos, aged 18-30years old. Data collection was carried out through oral examinations and self-administered questionnaires. The dental aesthetics of the students was assessed using the Aesthetic Component of the Index of Orthodontic Treatment (IOTN). Two oral health related quality (OHRQoL) of life instruments were used (1) the Shortened version of the Oral Health Impact Profile (OHIP-14) and (2) the Psychosocial impact of dental aesthetics questionnaire (PIDAQ). Data analysis included descriptive statistics, Chi-square tests and Kruskal-Walis test. **Results**: Statiscally significant relationships were established between the professionally assessed dental aesthetics of the students and both OHRQoL measures (PIDAQ and OHIP-14), particularly in the psychosocial domains of both scales. For the PIDAQ scale, the affected domains were dental self-confidence, psychological impact and aesthetic concern. While for the OHIP-14, four domains were affected, namely psychological discomfort, psychological disability, social disability and handicap. **Conclusion:** A statistically significant association exists between the dental aesthetics and specific aspects of the Oral health-related quality of life, of undergraduate students of the University of Lagos, particularly in the psychosocial domains.

***Keywords**: Dental Aesthetics, Quality of Life.*

INTRODUCTION

Facial aesthetics affect how people are perceived by society and how they perceive themselves. [1] The appearance of the teeth and an individual's smile are critical components of facial attractiveness.[2] Indeed, the oral-facial region is usually an area of significant concern for any individual because it draws the most attention from other people in interpersonal interactions and is the primary source of vocal, physical, and emotional communication.[3] The presence of a malocclusion (badly arranged teeth) can greatly impair the facial and dental aesthetics of an individual.

Malocclusion represents an important health problem worldwide.[4] Epidemiological surveys of malocclusion in several countries , have reported that this oral disorder is highly prevalent .[5] In the African continent, studies in several countries such as Kenya,[6,7] South Africa,[8,9] and Tanzania[10] have all recorded high prevalence values. In Nigeria in particular, high values have

also been reported, with prevalence values ranging from 49 to 88% .[11-17] These high prevalence values for malocclusion combined with the fact that it is treatable and preventable, underscore the need for it to be addressed as a public health problem.[18]

A malocclusion can be perceived differently by the affected person and a person's degree of awareness of their malocclusion might not be related to its severity.[19]Therefore, when evaluating the impact of a malocclusion, it is important to consider the different domains that can be affected and their relationships to the severity of the malocclusion.[20]Malocclusion not only affects oral function and appearance but also has economic, social, and psychological effects.[21] However, it is difficult to determine the precise impact of malocclusion on quality of life, as values attributed to dentofacial aesthetics vary according to cultural and social traditions.[1]
The general construct of quality of life originated in the field of general medicine and has been defined as "people's perception of their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectations, standards, and concerns" [22]or, more simply, as "a sense of well-being that stems from satisfaction or dissatisfaction with areas of life that are important to the individual".[23]The more specific concept of "oral health-related quality of life " has been defined as "a standard of health of oral and related tissues which enables an individual to eat, speak, and socialize without active disease, discomfort, or embarrassment"[24] or "the absence of negative impacts of oral conditions on social life and a positive sense of dentofacial self-confidence."[25]

Conditions affecting oral health, including malocclusion are highly prevalent and have consequences not only for physical and economic wellbeing, but can also impair quality of life by affecting function, appearance, interpersonal relationships, socializing, self-esteem and psychological wellbeing.[20] Understanding the physical , social and psychological impact of malocclusion on OHRQoL sheds light on the effects of malocclusion on people's lives and provides a greater understanding of the demand for orthodontic treatment beyond the measurement of clinical parameters. [26] In addition, since social and psychological effects are often the key motives for seeking orthodontic treatment, OHRQoL can be considered the best measurement for orthodontic treatment need and outcome.[27] Such research will be of great value to researchers, health planners and oral health care providers. [19]
Instruments measuring health-related quality of life address a patient's perspective of the impact of a medical condition on the subjective well-being and everyday functioning.[28] They use different response options to assess the impacts of conditions which range from frequency, severity, importance and the degree to which participants are bothered. Two commonly used OHRQoL instruments are The Psychosocial Impact of Dental Aesthetics Questionnaire (PIDAQ)[29] and the Oral Health Impact Profile (OHIP). [30]

Interestingly, most studies on the psychosocial aspects of malocclusion have been carried out in developed countries where people are more likely to have their basic needs met and orthodontic treatment is partially offered in public health services.[1] However, in underdeveloped and developing countries like Nigeria, the relationship between malocclusion, aesthetic impact and quality of life is largely unexplored. Furthermore, the use of these sociodental indicators would allow individuals with the greatest need to be a priority when financial resources are limited. [31]Thus, the aim of this study was to investigate the relationship between dentofacial aesthetics and the oral health-related quality of life of students of the University of Lagos. The findings from this study will be of benefit in planning for the provision of oral health care services, particularly orthodontic care, for students of the University of Lagos.

METHODS

Ethical approval for the study was obtained from the Health Research Ethics Committee (HREC) of the Lagos University Teaching Hospital, Idi-araba, Lagos and permission to carry out the study was also obtained from the Students' Affairs Office, of the University of Lagos. In addition, informed written consent was obtained from all students selected to participate in the study after the study had been fully explained to them.

This was a cross-sectional study involving undergraduate students of the University of Lagos aged 18-30 years, with no previous history of orthodontic treatment. A total of 420 undergraduate students of the University, from four randomly selected halls of residence (two male and two female halls) participated in the study.

Data was collected through self-administered questionnaires and dental examinations.Dental aesthetics was assessed using the Aesthetic Component of the Index of Orthodontic Treatment Need (IOTN).[32] This was used to assess the self-perceived and professionally assessed dental aesthetics of the students. Two instruments were used to assess the OHRQoL of the students namely the Psychosocial Impact of Dental Aesthetics Questionnaire (PIDAQ)[29] and the shortened version of the Oral Health Impact Scale (OHIP-14) [30].

The dental examinations and diagnostic criteria followed the World Health Organization (WHO) recommendations for oral health surveys.[33]A full intra-oral examination was carried out using a wooden tongue depressor, under natural light with the subject in sitting position on a chair.

Reliability

Two orthodontists were involved in determining the professionally assessed dental aesthetics of the students. Thus, to assess for inter-examiner reliability, both orthodontists independently examined ten students using the AC component of the IOTN, before the commencement of the study. A weighted kappa score of 0.7 was recorded showing good agreement, between both examiners. Intra-examiner reliability for both examiners were also recorded to give weighted kappa scores greater than 0.7' [34]

Pilot Study

A pilot study was carried out on twenty medical students in the College of Medicine, University of Lagos, Idi-araba; to test the methods and comprehension of instruments to be used in the study, prior to commencement of the study.

Statistical analysis

Participants were categorized into groups based on their IOTN AC scores. The AC scale of the IOTN was collapsed from a 10-point scale to a 3 point scale. Photographs 1 to 4 representing 'no need for treatment', 5 to 7 borderline need for treatment and 8 to 10 definite need for treatment, on aesthetic grounds.[31,32]Data entry and analysis was carried out using the Statistical Package for Social Sciences (SPSS) version 17, Chicago III . Descriptive statistics and Chi-square testswere used to analyze the data. Tests of normality showed that the data was not normally distributed. Therefore, mean PIDAQ subscale scores were compared using non-parametric tests (Kruskall-Wallis). The criteria for statistical significance was set at the 5% level.

RESULTS

Demographics

The final study sample was made up of three hundred and seventy-five subjects (375). The male students made up 53.3% (200), while the female students made up 46.7% (175). The records for 45 students, out of the initial sample of 420, were excluded from final analysis due to multiple missing entries in their questionnaires. Thus, the response rate was 89.26%. The mean age for the students was 21.16 + 2.65years and the modal age group was 18-20 years

Professionally assessed dental aesthetics of the Students

The professionally assessed dental aesthetics of the students revealed that 324 students (86.4%) had aesthetically acceptable teeth and 'no need' for orthodontic treatment (IOTN AC grades 1-4), 46 students (12.3%) had ' moderate need' for orthodontic treatment (IOTN AC grades 5-7) and 5 students (1.3%) had poor dental aesthetics (IOTN grades 8-10) and a 'definite need' for orthodontic treatment. The professional assessment showed a statistically significant difference ($p<0.05$), between the dental aesthetics for the males and females (Table 1).

Relationship between mean (PIDAQ) subscale scores and theprofessionally assessed dental aesthetics/orthodontic treatment need of the students.

A statistically significant difference ($p< 0.05$) was observed in the mean PIDAQ subscale scores of the students when analyzed according to their professionally assessed dental aesthetics. However, this statistically significant difference was only observed in three of the four PIDAQ subscales, with the 'Social impact' subscale showing no significant difference ($p>0.05$) between the aesthetic groups. This finding implies that there was a statistically significant difference in the oral health related quality of life of the students in the PIDAQ subscales of "Dental Self Confidence', 'Psychological Impact' and Aesthetic Concern, when analyzed based on their professionally assessed dental aesthetics and orthodontic treatment need (Table 2).

Relationship between OHIP- 14 scores and the professionally assessed dental aesthetics of the students.

A statistically significant association was observed between the following OHIP-14 measures and the professionally assessed dental aesthetics of the students: 'being self-conscious'; 'having an unsatisfactory diet' ‘being irritable with other people’ and 'finding that life in general is less satisfying' Thus, the OHIP-14 domains of psychological discomfort, physical disability, social disability andhandicap,were significantly associated with the professionally assessed dental aesthetics of the students. Conversely, the other domains of functional limitation, physical pain, psychological disability and social disability were not significantly associated with the professionally assessed dental aesthetics and orthodontic treatment need of the students. (Table 3).

DISCUSSION

It is now generally accepted that the measurement of oral health related quality of life is an essential component of oral health surveys, clinical trials and studies evaluating the outcomes of preventive and therapeutic programs intended to improve oral health. Thus, assessment of oral health-related quality of life has an important role to play in clinical practice.[35] Indeed, clinicians are increasingly placing more emphasis on patient based evaluations of health-related quality of life.[36] This might be particularly important in cosmetic and elective treatments.[37]Thus, of all the dental treatments that require the use of oral health-related quality of life measures, the treatment of malocclusion, which has a large psychosocial component, calls for the use of these measures.[38]

The orthodontists' (professional) assessment of the students’ dental aesthetics revealed that 13.6% of the students had a need for orthodontic treatmentwith only 1.3% recording ‘definite need' for treatment. This prevalence is low compared to other Nigerian studies in Ile-Ife[39]and Rivers State[40] which reported overall treatment need and definite treatment need values of 55.2%, 11.0% and 35.1%, 17.6%; respectively. It is also lower than that reported in a Jordanian population. (27.1%, 7.3%).[41]This indicates a relatively low orthodontic treatment need among undergraduate students of the University of Lagos. However, the percentage of students in this study with a definite need for orthodontic treatment (1.3%) is closely related to that reported for

a Peruvian University population of 1.8%.[42] It is instructive to note that significant gender differences were observed in the orthodontists' (professional) assessment of the dental aesthetics of the students. The female students recorded a significantly greater need for treatment than the males. However, other studies have reported the absence of significant gender differences.[43,44]

Two oral health-related quality of life scales, which have been previously validated and tested in this environment, were selected for use in this study.[39,45]The PIDAQ scale which is a condition specific scale for assessing orthodontic specific aspects of quality of life were chosenbecause it was specifically designed to assess the impact of dental aesthetics on the psychosocial aspects of the quality of life of young adults.[29] and the OHIP-14 a generic scale for assessing the impact of oral health on daily activities and functions[30] . Bearing in mind that the aim of this study was to assess the relationship between dental aesthetics and quality of life, the PIDAQ scale was considered a very important tool for this study. On the other hand the OHIP-14 was chosen for this study because it is a generic scale which covers all aspects of oral health -related quality of life.[30] Thus, its inclusion in this study was to ensure that as much as possible all aspects of the OHRQoL of the students were covered in study.

The dental aestheticsof the students differed significantly ($p<0.05$) in the PIDAQ subscale scores for Dental Self-confidence, Psychological Impact and Aesthetic Concern. This implies that the different categories of dental aesthetics/orthodontic treatment need, exhibited significantly different levels of dental self-confidence; psychological impact and aesthetic concern among the students. However for the 'Social Impact' subscale, no significant difference was observed.'Dental self-confidence' was the most affected domain in the PIDAQ subscales for professionally assessed dental aesthetics, ($p<0.000$). Similar findings have also been reported in closely related studies.[29,39] Dental self-confidence' indicates the level of satisfaction or dissatisfaction with the appearance of one's dentition, and aims to measure the influence of dental aesthetics on the self-image of an individual.[29] The appearance of the mouth and smile play an important role in assessment of facial attractiveness, which undoubtedly contributes to self-concept and self-esteem.[46] Individuals who are aware of their malocclusion may focus increasingly on it and may even develop anxieties. It is also possible for them to exhibit increased private self-consciousness which may predispose to self-criticism and self-dissatisfaction manifesting as low dental self- confidence.[39] Furthermore, the results from this study suggest a trend of decreasing dental self-confidence with increasing levels of altered dental aesthetics (poorer dental aesthetics). This has also been reported in previous studies,[29,47] corroborating the fact that a set of well aligned teeth may be associated with more favorable oral-health attitudes, and a higher degree of satisfaction regarding dental attractiveness resulting in better self-concept. [48]

With respect to the OHIP-14 measure, it is instructive to note that 'being self-conscious', 'having an unsatisfactory diet', 'being irritable with other people' and 'finding that life in general is less satisfying' were areas of life of the students that were significantly associated with their professionally assessed dental aesthetics. These correspond with the OHIP-14 domains ofpsychological discomfort, physical disability, social disability and handicap, respectively. The association observed with 'being self-conscious' agrees with the finding of Klages et al,[49] who found that young adults with more severe forms of malocclusion had higher self-consciousness scores. Furthermore, the statistically significant association observed between the social disability domain of the OHIP-14 and the professionally assessed dental aesthetics of the students may not be unconnected with the fact that dental and facial appearance could influence the social activities and the success of interpersonal relationships.[49]

The findings from this study, reinforce previous reports which suggest that the most significant impact of quality of life expresses itself in the psychosocial domain rather than in dissatisfaction with function.[38] Psychometric scales reveal that questions related to emotional and social domains, including aspects such as shyness, embarrassment, being upset, and avoidance of smiling or laughing , are more relevant to an orthodontic patient.[38] There is ample evidence that patients focus primarily on esthetic and social aspects of OHRQoL as a motive for seeking orthodontic treatment; this is true for children as young as eight and for adult patients.[3] and these findings are also reflected in this study carried out in a young adult population. Thus, it is important for the general dentist and orthodontist to listen carefully to each patient's understanding of his or her malocclusion and its impact on quality of life domains.[3] Indeed, additional oral health related quality of life information acquired from the subject, would enhance normative orthodontic treatment need assessments. [27]All these would go a long way in improving the quality of treatment planning and ultimately treatment outcomes, for the orthodontic patient in this environment.

CONCLUSION

These results highlight the fact that a statistically significant association exists between the dental aesthetics and specific aspectsof the Oral health-related quality of life of the undergraduate students of the University of Lagos, particularly in the psychosocial domains. These findings will be of benefit in the provision of oral health care services, particularly orthodontic care, to students of the University of Lagos.

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Table 1: Professionally- assessed dental aesthetics and orthodontic treatment need of the students using the Aesthetic component of the IOTN

VARIABLE	**FREQUENCY**		
Professionally Assessed Dental aesthetics/ Orthodontic Treatment Need **Aesthetic Component of IOTN**	**Male n (%)**	**Female n(%)**	**Total n(%)**
No Need for treatment	183 (91.5)	141 (80.6)	324 (86.4)
Moderate Need for treatment	16 (8.0)	30 (17.1)	46 (12.3)
Definite Need for treatment	1 (0.5)	4 (2.3)	5 (1.3)
Total	200 (100)	175 (100)	375 (100)

Chi square = 9.883, p=0.007. IOTN: Index of orthodontic treatment need.

Table 2: Relationship between mean (PIDAQ) Psychosocial Impact of Dental aesthetics Questionnaire) subscale scores and the professionally assessed dental aesthetics/orthodontic treatment need of the students.

PIDAQ subscale	Aesthetic Component of IOTN ('Professionally assessed')			P value
	No need for treatment Mean (sd)	Borderline Need for treatment. Mean (sd)	Definite Need for treatment Mean (sd)	
Dental Self Confidence	14.7 (6.0)	10.7(6.0)	8.8((5.0)	0.000*
Social Impact	5.4(6.5)	6.8(7.2)	6.8 (8.8)	0.305
Psychological impact	7.9(5.6)	9.8(6.2)	1.2(3.4)	0.047*
Aesthetic concern	1.5(2.6)	2.8(3.5)	2.6(3.2)	0.003*

***p< 0.05, Kruskal-Wallis**

Table 3A: The relationship between OHIP- 14 scores and the professionally assessed dental aesthetics of the students.

	OHIP 14 Daily Activity	**No Need**	**Moderate need**	**Definite need**	**Chi-square P value**
	Functional Limitation				
1.	Had problems pronouncing works				
	-No Impact n (%)	261(80.6)	32(69.6)	3(60.0)	4.018
	-Impactn (%)	63(19.4)	14(30.4)	2(40.0)	0.134
2	Felt your sense of taste has worsened				
	-No Impact n (%)	272(84.0)	38(82.6)	3(60.0)	2.075
	-Impact n (%)	52(16.0)	8(17.4)	2(40.0)	0.354
	Physical Pain				
3.	Had a painful aching in the mouth				
	-No Impact n (%)	159(49.1)	14(30.4)	2(40.0)	5.713
	-Impactn (%)	165(50.9)	32(69.6)	3(60.0)	0.057
4.	Found it uncomfortable to eat any food				
	-No Impact n (%)	197(60.8)	25(54.3)	3(60.0)	0.699
	-Impactn (%)	127(39.2)	21(45.7)	2(40.0)	0.705
	Psychological discomfort				
	Have you been self conscious				
5.	-No Impact n (%)	189(58.3)	21(45.7)	0(0.0)	9.079
	-Impactn (%)	135(41.7)	25(54.3)	5(100.0)	0.011*
6.	Felt tense				
	-No Impact n (%)	253(78.1)	32(69.6)	2(40.0)	5.395
	-Impactn (%)	71(21.9)	14(30.4)	3(60.0)	0.067
	Physical Disability				
7.	Had an unsatisfactory diet				
	-No Impact n (%)	246(75.9)	36(78.3)	1(20.0)	8.539
	-Impactn (%)	78(24.1)	10(21.7)	4(80.0)	0.014*
8.	Had to interrupt meals	266(82.1)	33(71.1)	3(60.0)	4.120
	-No Impact n (%)	58 (17.9)	13(28.3)	2(40.0)	0.127
	-Impactn (%)				

*$p<0.05$

Table 3B: The relationship between OHIP- 14 scores and the professionally assessed dental aesthetics of the students

	OHIP 14 Daily Activity	**No need**	**Moderate Need**	**Definite Need**	**Chi square P value**
9.	**Psychological Disability** Found it difficult to relax				
	-No Impact n (%)	278(85.8)	34(73.9)	5(100.0)	5.282
	-Impactn (%)	46(14.2)	12(26.1)	0(0.0)	0.071
10.	Have been a bit embarrassed				
	-No Impact n (%)	257(79.3)	33(71.7)	2(40.0)	5.560
	-Impactn (%)	67(20.7)	13(28.3)	3(60.0)	0.062
11.	**Social Disability** Have been irritable with other people				
	-No Impact n (%)	249(76.9)	28(60.9)	3(60.0)	6.016
	-Impactn (%)	75(23.1)	18(39.1)	2(40.0)	0.049
12.	Have difficulty doing usual jobs				
	-No Impact n (%)	298(92.0)	42(91.3)	4(80.0)	0.944
	-Impactn (%)	26(8.0)	4(8.7)	1(20.0)	0.624
13.	**Handicap** Felt life in general is less satisfying				
	-No Impact n (%)	247(76.2)	31(67.4)	1(20.0)	9.528
	-Impactn (%)	77(23.8)	15(32.6)	4(80.0)	0.009*
14.	Have been totally unable to function				
	-No Impact n (%)	311(96.0)	43(93.5)	5(100.0)	0.847
	-Impactn (%)	13(4.0)	3(6.5)	0(0.0)	0.655

*$p<0.05$

HOLDAWAY'S ANALYSIS OF THE NOSE PROMINENCE OF AN ADULT NIGERIAN POPULATION

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ABSTARCT

Background: Facial beauty is a function of harmonious balance among all parts of the face and the nose plays a dominant role in this because of its location exactly in the middle of the face. Therefore, an evaluation of nasal form and its position relative to other facial structures should play an important part in the assessment of patients before orthognathic surgery, rhinoplasty or orthodontics. **Aim**: To establish normative values for the Nose prominence of an adult Nigerian population using Holdaway's soft tissue cephalometric analysis. **Methodology**: Lateral cephalometric radiographs of 100 adults aged 18-25 years, with normal occlusion and a harmonious facial appearance were analyzed. The nose prominence was assessed using Holdaway's analysis. Twenty radiographs randomly selected, were retraced to assess for errors. Data analysis included descriptive statistics and student's t-tests using Statistical Package for Social Sciences (SPSS). **Results**: The mean value recorded for the nose prominence of the study population was 3.59mm + 3.49, with a range of -5.0mm to 15.0mm. Mean values obtained for females was 3.75mm + 2.90 and males 3.91mm +3.69. No statistically significant gender difference was observed. ($p>0.05$). **Conclusion**: Normative values were established for the nose prominence of an adult Nigerian population. The values obtained for Nigerians in this study are comparatively lower than that reported for other populations. These values would aid in treatment planning for orthognathic surgery, rhinoplasty and orthodontics in Nigerians.

Keywords:*Cephalometrics, Holdaway's Analysis, Nose prominence*

INTRODUCTION

Facial beauty is a function of harmonious balanceamong all parts of the face and the nose plays a dominant role in thisbecause of its location exactly in the middle of the face.[1]Therefore, an evaluation of nasal form and its position relative to other facial structures should play an important part in the assessment of patients before orthognathic surgery, rhinoplasty or orthodontics.[2]

The nose may be evaluated by direct clinical measurements (morphometry),[3-7] by photogrammetry,[8-10] by radiographs (cephalometry)[2,11] or more recently by three-dimensional (3D) stereo-photogrammetric systems [12, 13].Morphometry and photogrammetry both offer cost effective means of carrying out anthropometric studies, however, cephalometry offers a major advantage over these methods in that it is capable of simultaneously imaging the soft tissue profile and the facial skeleton.[2]While three dimensional (3D) scans can be used to obtain very accurate anthropometric measurements, its use is limited because of the huge cost involved in acquiring such systems.

A number of nasal morphometricand photogrammetricstudies havebeen carried out in the Nigerian population.[3-7,14,15]However, there is currently a paucity of data on cephalometric studies of the nasal profile of theNigerian population. This data if available would aid in treatment planning for orthodontics, rhinoplastyand orthognathic surgery.

Holdaway's soft tissue analysis has been used in several studies to report the cephalometric soft tissue findings of different ethnicities, in addition to the comparison of these findings to established Holdaway norms.[16-26]In his analysis, Holdaway described the Nose prominence as the distance between the tip of the nose and a perpendicular line drawn to the Frankfort plane from the vermillion border of the upper lip.[16]

The purpose of this study was to establish normative values for the Nose prominence of an adult Nigerian population using Holdaway's soft tissue cephalometric analysis.

METHODS

The subjects were made up ofsecond year to final year medical, dental and pharmacy students of the College of Medicine, University of Lagos, Idi-araba, Lagos, Nigeria. The sample comprised 100 subjects (56males and 44 females); mean age 21.63 years) who met the selection criteria. Ethical approval for the study was obtained from the ethical committees of the College of Medicine, University of Lagos and the Lagos University Teaching Hospital, Idi-araba, Lagos. In addition, informed written consent was obtained from each subject after the nature and purpose of the radiographs had been explained to them. The radiographs used in this study had been used in a previous study to assess the horizontal lip relationships of the study sample.[27]

The lateral cephalometric radiographs were manually traced on 0.003-mm matte acetate sheets (MASEL, 2034-007, AR-MED Ltd, UK), with a 0.5mm lead pencil.

Based on the definition by Holdaway,[16]the Nose prominence was described as the distance from a line perpendicular to Frankfort Horizontal and running tangent to the vermillion border of the upper lip, to the tip of the nose (Figure 1)Thelinear measurement was made with a graduated metric ruler to the nearest 0.5mm.

Definition of the landmarks and reference plane (Frankfort plane) necessary for measuring the Nose prominence**:**

- Pronasale (Pn):The most prominent or anterior point of the nose (tip of the nose).
- Labralesuperius (Ls):The most anterior point of the upper lip.
- Orbitale (O): The lowest point of the infra-orbital margin, where two orbitalia were visible, a point midway between the two was used.
- Porion (P): The uppermost point of the bony external auditory meatus.
- Frankfort plane (FP): This is a straight line passing through the porion and orbitale.

Statistical Analysis

The Statistical package for social sciences (SPSS) version 17, Chicago III, was used for analyzing data. Descriptive statistics of mean and standard deviation were obtained. Students' t-test was used to determine the gender differences at a significance level of $p<0.05$.

To assess errors in the cephalometric tracing, 20 randomly selected lateral cephalograms were retraced after an interval of 7 days. The error was then calculated by using Dahlberg's equation[28].Paired t-tests were also carried out between the initial and repeat measurements to determine the significance of any error. The level of significance was also set at $p<0.05$.

The methodological cephalometric tracing error calculated using Dahlberg's equation[28] was found to be 0.74mm and this falls within the normal range reported by Baumrind and Frantz,[29]for linear measurement errors in cephalometric studies, which is 0.43mm to 0.86mm. In addition, a paired t –test between the initial sample and twenty randomly selected radiographs showed no statistically significant difference between the first and second tracings ($p>0.05$).

RESULTS

A total of 100 subjects, 44% males and 56% females, aged 18-25 years with a mean age of 21.63 + 2.04 years were seen.

The mean value recorded for the nose prominence in this study was 3.59mm + 3.49, with a range of -5.0mm to15.0mm. The mean value obtained for females was 3.75mm + 2.90 and males 3.91mm+3.69. There were no statistically significant gender difference observed ($p>0.05$).
Thus, male and female data were pooled together, in comparing the mean nose prominence obtained in this study, with that reported for other populations from other studies. (Table 1)

DISCUSSION

The nose, a striking feature of the human face, is regarded by some clinicians as the keystone of facial aesthetics.[30, 31] It has also been reported that the perceptions of and attitude to, facial appearance are influenced by the form of the nose and its relationship to other parts of the soft tissue profile. [32] The racial and ethnic features of each patient's nose are dependent on the underlying bony and cartilaginous skeletal frameworks together with the skin and the soft tissue envelopes. These features have genetic basis but are also influenced by environmental factors such as trauma, ageing, nutrition and surgery. [33]

The importance of a thorough assessment of nasal form in treatment planning for orthodontic treatment or surgical procedures such as orthognathic surgery and rhinoplasty cannot be overemphasized. Some forms of therapy, may either directly or indirectly alter the form of the nose and, thus facial appearance. For example orthodontic treatment to reduce protruding incisors can lead to lip changes that increase the relative prominence of the nose. [2,34] Mandibular surgery may also affect the relative prominence of the nose because of changes in the soft tissue chin and lower lip. [2, 31] Maxillary and nasal surgery, however are more likely to affect the form of the nose directly.[30,31]
Nose prominence is considered to be a recognizable individual facial characteristic.[26] Its magnitude would affect treatment-planning decisions as it influences the presentation of adjacent circum oral and facial structures.[26] This study was carried out to provide baseline data on the nose prominence values of adult Nigerians, using Holdaway's analysis. Holdaway's analysis was chosen as it has been widely used in different studies to assess the soft tissue profiles of different populations. [16-25] Thus, providing a greater basis for comparing the findings from this study to that reported for other populations.

No significant gender differences were observed in the nose prominence of the adult Nigerians in this study. A similar finding was reported in Indians by Mehtaet al,[24]. However, studies carried out in Turkish, [17] and Persian,[23] populations have reported marked sexual dimorphism with the males having a larger nose prominence than females.

According to Holdaway,[16] nose prominence has an acceptable range of 14 to 24 mm. Holdaway suggested that noses less than 14 mm are small, and those above 24 mm are large or prominent, with respect to a white Caucasian population. However, the mean value for the nose prominence recorded for Nigerians in this study was 3.59mm, thus indicating that Nigerians have a significantly smallernose prominence in comparison to Caucasians. Flynn et al,[35] also reported significantly lower nasal projection (horizontal distance from the subnasale to the pronasale) in Black American adults (11. 9mm) as compared to the white population (15.7mm). The meanvalue recorded for Nigerians in this study is also much lower than that recorded for several other populations as shown in Table 1. The population whose nasal prominence was closest to that of Nigerians were the Chinese with a value of 6mm as reported by Lew et al.[18] The values recorded for several other populations such as the Japanese[19](14.54mm), Turkish[17](18.74mm) and Pakistanis[21](19.36mm), amongst others, were all markedly higher than that recorded for Nigerians in this study (Table1) . Unfortunately, there is currently a paucity of published data on soft tissue cephalometric analysis carried out in African populations, particularly with respect to

Holdaway's analysis.Thus, making it difficult to compare the nasal prominence for Nigerians with that for other African populations.

A major limitation of Holdaway's nose prominence as a soft tissue cephalometric parameter for assessing the nasal profile is that it does not provide a detailed cephalometric analysis of the nose. More detailed cephalometric analyses of the nose have been described by other authors.[1,2,11]Thus, there will be need for future studies to assess the nasal profile of adult Nigerians using some of these analyses.However, the nasal prominence values obtained for Nigerians in this study will be of use in treatment planning and has produced baseline values for comparison with other populations.The findings from this study further reinforce the findings from different studies carried out in different ethnic groups, which show that soft tissue features are specific for a given ethnic group or population.[16-26]Therefore population specific norms should be used as reference values for treatment planning purposes.

CONCLUSION

The mean nasal prominence values obtained for Nigerians in this study was 3.59mm and no significant gender differences were observed. The values established for Nigerians were markedly lower than that reported for Caucasian and other populations. These values will be of benefit for treatment planning for Nigerians undergoing orthodontic treatment or surgical procedures such as orthognathic surgery and rhinoplasty.

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TABLE 1
A Comparison of the Nose Prominence Recorded in this Study with that Reported for other Populations by Different Authors

Author(S)	Year of Publication	Population Studied	Nose Prominence(mm)
Holdaway[16]	1983	Caucasian	14-24
Lew et al[18]	1992	Chinese	6.00
Alcalde et al[19]	2000	Japanese	14.54
Basciftci et al[17]	2003	Turkish	18.74
Al-Gunaid et al[20]	2007	Yemeni males	16.70
Hammed et al[21]	2008	Pakistanis	19.36
Al Azemi et al[22]	2008	Kuwaitis	14.25
AlTaki et al[23]	2009	Persian adults	16.72
Mehta et al[24]	2010	Indians	13.38
Hussein et al[25]	2011	Palestinians	19.24
ALBarakati[26]	2012	Saudi Arabian	13.46
Present Study	**2014**	**Nigerians**	**3.59**

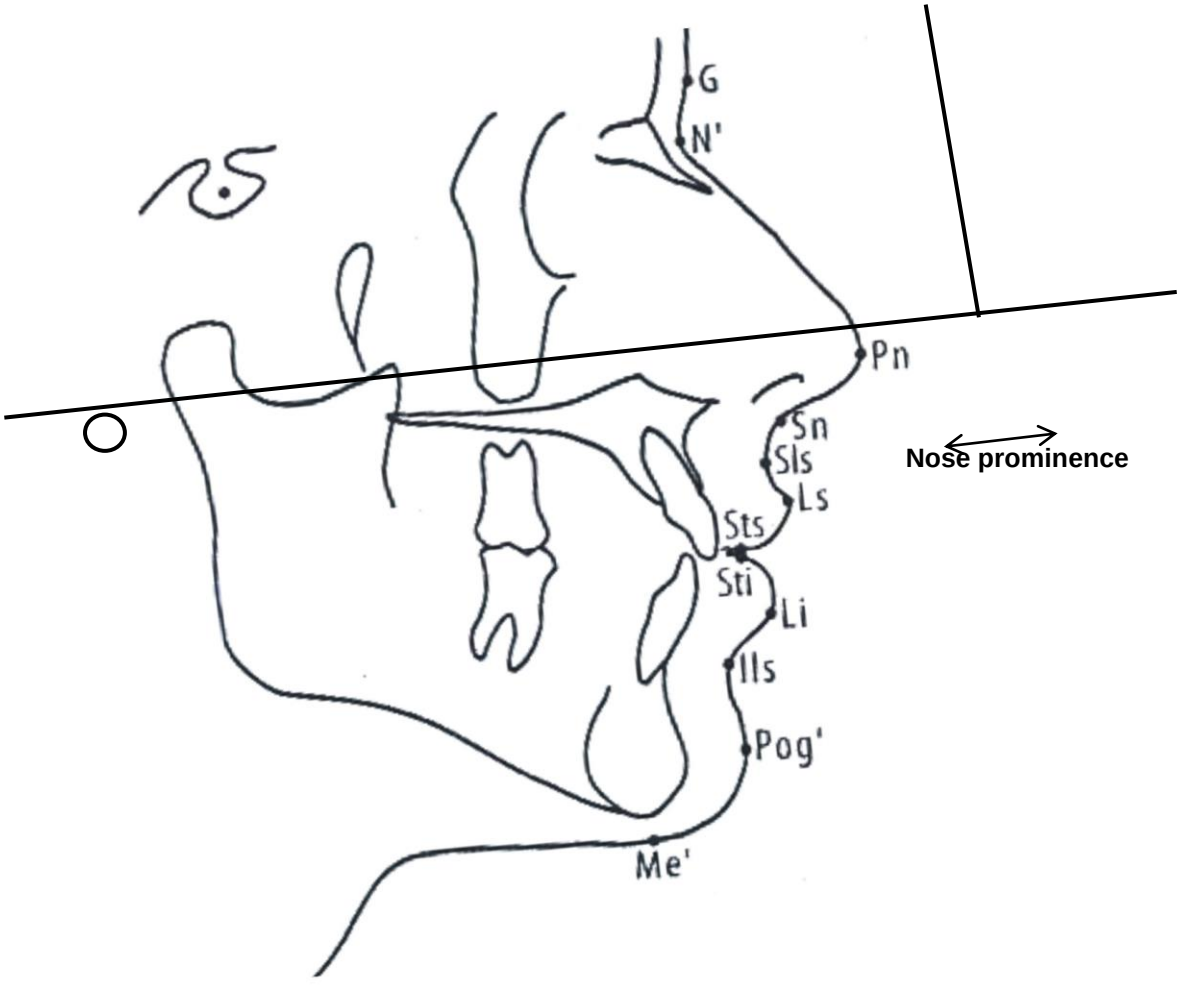

Figure 1: Nose prominence.

ASSOCIATION BETWEEN PERIODONTAL DISEASE AND SYSTEMIC CONDITIONS-A SURVEY AMONG INTERNAL MEDICINE RESIDENTS IN NIGERIA

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ABSTRACT

Background: There is growing evidence that periodontal diseases (PD) may be a source of systemic inflammation that impacts overall health. Due to the grossly inadequate dentist to population ratio in Nigeria, many patients with dental problems tend to seek oral health care from medical doctors than dentists. Hence, there is the need for medical doctors to be well informed about the bilateral link between periodontal and systemic diseases to ensure the timely referral of such patients. **Objectives:** To determine the knowledge of the association between periodontal diseases and systemic conditions among internal medicine residents and to assess their attitude towards periodontal health in their patients. **Methods:** In a cross-sectional survey, self-administered questionnaires were distributed to 150 Internal Medicine Residents attending the Faculty of Internal Medicine 2014 Update Course organized by the National Postgraduate Medical College of Nigeria. They were representative of the 6 geopolitical zones in Nigeria. The questionnaire assessed their knowledge of PD, its bidirectional relationship with systemic diseases and their attitude to periodontal health among their patients. Data was analyzed with Epi Info Software Version 3.5.4 and presented as mean and frequencies. **Results:** A total of 123 residents returned their questionnaires, while 109 filled them properly. Males represented 67.9%. Mean age was 33.1 years. The Television was their most common source of information on oral health (59.4%). Only 11.2% correctly knew gum bleeding to be the earliest sign of PD. PD was correctly associated with smoking (91.7%), HIV/AIDS (79.8%), diabetes mellitus (78%) and pregnancy (34.9%). Also, PD was correctly identified as a risk factor for coronary heart disease (45.9%), stroke (43.5%), hospital acquired pneumonia (53.2%), diabetes mellitus (13.8%) and pre-term babies (11%). Majority (90.9%) felt it was important to assess their patients regularly for periodontal disease. **Conclusions:** The knowledge of PD among the internal medicine residents was poor, while their knowledge of PD as a risk factor for some systemic conditions was inadequate. They had a positive attitude towards periodontal health care for their patients.

Keywords: *Knowledge, Periodontal disease, Internal Medicine Residents, Nigeria*

INTRODUCTION

Periodontal diseases are highly prevalent oral diseases that contribute to the global burden of chronic diseases and as such constitute a major public health problem.[1,2] The importance of oral health to general wellbeing of a person (periodontal health being a key component) has been reiterated.[3] Most periodontal diseases are inflammatory in nature and are initiated by plaque biofilm which results in gingivitis, the milder form of the disease. The earliest sign of gingivitis is bleeding from the gum especially during tooth brushing. Periodontitis, on the other hand, is an interaction between the host immune response and the infectious agents in biofilms and results in attachment loss and tooth loss if untreated early.[4,5] These diseases can be readily reversed by effective plaque control measures such as daily tooth brushing and the use of interdental cleaning aids such as dental floss.[6]

Of great importance is the growing body of evidence in literature in the last two decades reporting a bilateral link between periodontitis and a wide range of systemic conditions such as cardiovascular disease,[7] diabetes mellitus,[8] and poor pregnancy outcomes.[9] It has been suggested that periodontitis may be a source of systemic inflammation that impacts overall health.[10] Although, periodontal diseases are preventable, their successful prevention and management depends to a large extent on high awareness and good knowledge of their causes, early symptoms and control.[11]

In Nigeria, the level of awareness of oral diseases among the general population has been documented as being low.[12] This may have serious consequences for patients with medical problems who have oral-related problems such as periodontal disease. With the grossly inadequate dentist to population ratio in Nigeria[12] patients are more likely to seek oral health care from medical professionals rather than dentists. This has been documented by researchers in other countries. [13-15]

Furthermore, in developing countries, there has been an epidemiological transition in the burden of global diseases with a shift from infectious diseases to non-communicable diseases such as hypertension, cardiovascular diseases, and diabetes in the last 40 years.[16] According to the World Health Organization [17,18] these chronic diseases currently account for about 40% of the global burden of diseases which is expected to rise to 60% by year 2020. The implication of this is that there would be more patients with these chronic systemic illnesses that will present with periodontal and other oral problems to physicians.

Hence, medical doctors, especially physicians must be well informed about periodontal disease as well as their bilateral link with systemic conditions to ensure best practices regarding patient care. Studies done in developed[19] and developing countries[20-23] have shown varying outcome with some reporting a high level of awareness[20] while others reported limited awareness among doctors.[21,23]

Very few studies have reported the knowledge of physicians specialized in internal medicine about periodontal diseases as well as its bilateral association with systemic conditions [24] and the attitude of medical doctors in Nigeria towards the referral of their patients to the dentists.[25] A recent study in Nigeria found that medical doctors from the South West had poor perception of the influence of dental conditions on the general state of their patients' health.[24]

Our study focused on internal medicine residents in Nigeria as they are among the first to see large number of patients at the clinics. Our aim was to assess their knowledge of periodontal disease, its association with systemic conditions and their attitude towards the periodontal health of their patients.

METHODS

Study location and design

This was a cross-sectional survey conducted among internal medicine residents from several residency training institutions in Nigeria attending the Faculty of Internal Medicine 2014 Update Course organized by the National Postgraduate Medical College of Nigeria.

Data Collection

Self-administered questionnaires were distributed to all consecutive attending resident doctors who consented to participate in the study. The questionnaire had 3 sections: Section A assessed their demographic characteristics, geo-political zone, number of years since graduation from medical school, number of years in the residency training program, as well as their source of information on oral health.

Section B assessed their knowledge of periodontal disease, its bidirectional relationship with different systemic conditions and medications and their opinion on the adequacy of dental education for undergraduate medical students in Nigeria. Some of the questions had 'Yes' 'No' and 'Not sure' options. Four questions were asked to assess the knowledge of the respondents about periodontal disease and each question had one correct response. A correct response was scored 25%, while wrong response was scored 0%. Thus, the level of knowledge had scores ranging from 0 to 100%. This was regrouped into 2 categories: those with inadequate level of knowledge (0-50%) and those with adequate level of knowledge about periodontal disease (51-100%). These criteria were based on a previous study.[26] Their knowledge of the different specialties of Dentistry in Nigeria was also assessed.

In Section C, their attitude towards the periodontal health of their patients was assessed using a Likert scale. The study was approved by the Research and Ethics Committee of the Lagos University Teaching Hospital. The data was analyzed with Epi Info Software Version 3.5.4 package and the results presented as means and frequencies. The level of significance was set at $p<0.05$.

RESULTS

Out of 150 questionnaires distributed, 123 returned their questionnaires, giving a response rate of 82%. Of these, 109 filled their questionnaires properly. Table 1 shows their socio-demographic characteristics. Males represented 67.9% with an overall mean age of 33.1 years. The most common sources of oral health information for the respondents were television (59.4%), undergraduate training in medical school (51.9%) and dentists/other dental health professionals (50%) (Table 2).

The questions testing their knowledge of periodontal disease are shown in Table 3. Although, 70.1% knew the term 'periodontal disease' to be the same as gum disease, only 12 (11.2%) knew gum bleeding to be its earliest symptom.

Overall, only 28.4% of the resident doctors had adequate knowledge of periodontal disease (Figure A).

Their knowledge of periodontal disease was not associated with age ($p=0.298$), gender ($p=0.247$), designation ($p=0.372$), geo-political zone (0.669), number of years of graduation from medical school ($p=0.494$) and number of years of residency training ($p=0.290$).

More than 50% of the residents were aware of a possible link between periodontal disease and smoking, HIV/AIDS, diabetes, leukemia, stress and CKD (Table 4).

Periodontal disease was correctly identified as a risk factor for hospital acquired pneumonia (53.2%), coronary heart disease (45.9%), Stroke (43.5%), peripheral arterial disease (28.4%) and pre-term/low birth weight babies (11%). See Table 5.

Regarding medications associated with periodontal disease, more than 50% of the residents correctly selected phenytoin. Their knowledge regarding cyclosporine, nifedipine and oral contraceptives is shown in Table 6.

The attitude of resident doctors towards their patients' periodontal health is shown in Table 7. Ninety nine (90.9%) agreed that it was important to assess their patients regularly for periodontal disease, while 88 (80.7%) agreed that they should refer their patients for routine dental check-up.

DISCUSSION

The present study was predicated on the fact that periodontal disease has been established to be one of the novel/emerging risk factors in many systemic diseases.[27] Since physicians manage these conditions frequently, there is need for them to have adequate knowledge of these associations. The major findings of our study among the resident doctors in Nigeria were the inadequate knowledge of periodontal disease, fair to good knowledge of the association between periodontal and some systemic diseases/conditions and a limited awareness of periodontal

disease as a risk factor for many systemic diseases. The respondents however had a positive attitude towards referring their patients for regular dental checkup.

Few (28.4%) of the resident doctors had adequate knowledge of the periodontal diseases (the meaning, cause, earliest sign and the best form of prevention). This contrasts with the study by Shar et al.,[20,23] in which majority had good knowledge of periodontal disease. The difference could be possibly explained by the possible low level of exposure of the respondents in the present study to dental health education during their undergraduate training in medical school (51.9%) and continuing medical education (23.1%) during their postgraduate training.

The fact that more than 50% of the residents in this study knew of an existing association between periodontal diseases and some common systemic conditions/diseases such as smoking, HIV/AIDS, diabetes, leukemia, stress and chronic kidney disease was encouraging. An earlier study among Nigerian doctors revealed that majority (81.6%) knew of some link between diabetes and gum disease which supports our findings concerning diabetes.[28] The knowledge of periodontal disease as a risk factor for diabetes was poor (13.7%) in the present study. This indicates the need to raise the level of awareness among these group of doctors who are on their way to becoming the next set of specialists. Moreover, the rising prevalence of diabetes in this environment necessitates a call for better collaboration between physicians and dentists to improve the patient's care who is at the receiving end of the clinical practice whether good or poor. Also noted was their poor knowledge regarding common conditions such as pregnancy (38.9%) and erectile dysfunction (16.5%). Previous studies have reported a general apathy and lack of knowledge of periodontal diseases and periodontitis as a risk factor for infertility among Nigerian specialists.[29]

This study also highlighted the poor knowledge of the resident doctors about the side effects of some commonly prescribed medications such as nifedipine. It is one of the commonest antihypertensive drugs and had gingival enlargement as one of its most important effects in dentistry.[30] Resident doctors ought to be familiar with some of these likely complications that may occur and manage them promptly. Further assessment of the residents in this study revealed poor knowledge of periodontal disease as a risk factor for systemic conditions such as coronary heart disease, stroke, peripheral arterial disease, diabetes/poor glycemic control and preterm low birth weight babies (11-45.9%). This poor awareness has been reported in other study.[10,22] The poor knowledge demonstrated here is alarming owing to current knowledge of the impact of periodontitis on systemic diseases as earlier highlighted. It has been reported that bacterial products such as lipopolysaccharides released into the systemic circulation during periodontal infections induce a major vascular response in many systemic conditions such as coronary heart disease, diabetes mellitus, stroke, hospital acquired pneumonia and preterm labor (low birth weight babies).[27] Periodontal disease may thus act as an independent risk factor. Fortunately, periodontal disease is a modifiable one and can be controlled if detected early.

The limited knowledge displayed in this study by the residents warrants the need for inclusion of more dental topics in undergraduate as well as postgraduate programs in medical curriculums in Nigerian undergraduate and postgraduate institutions.

The positive attitude displayed by the physicians towards referring their patients for dental checkup is a step in the right direction which would further improve the referrals of patients with systemic diseases by physicians to dentists/periodontologist for dental care and thus reduce the burden of systemic diseases. It may also encourage physicians to carry out simple oral examinations which may go a long way in detecting common oral and periodontal diseases. This is a good way to encourage the medical-dental collaboration which is very much needed in the Nigerian health system.

CONCLUSION

Our study found that resident doctors in Nigeria had inadequate knowledge of periodontal disease, and its importance as a risk factor for many systemic diseases. They however, had a positive attitude towards referring their patients for regular dental checkup. Inclusion of dental health courses in the undergraduate and postgraduate curriculum is recommended.

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Table 1: Socio-demographic characteristics of the Resident doctors

Characteristics	n (109)	%
Age (years)		
26-35	82	75.2
36-45	27	24.8
Mean (±SD)	33.1 (±4.0)	
Sex		
Male	74	67.9
Female	35	32.1
Designation		
Junior Registrar	91	83.5
Senior Registrar	18	16.5
Geopolitical zones for Residency training program (n=84)		
South West	29	34.5
South East	14	16.7
South South	13	15.5
North Central	16	19.0
North West	7	8.3
North East	5	6.0
Non response	25	
Number of years of graduation from medical school (n=107) Mean (±SD)	7.4 (±2.9)	
Number of years in residency training program (n=108) Mean (±SD)	2.7 (±1.7)	

Table 2: Sources of information about Oral health

Main source of information	n	%
Television	63	59.4
Undergraduate training in medical school	56	51.9
Dentists/Other dental health workers	54	50.0
Books/Journals	43	39.8
Radio	34	31.8
Family/Friends	30	28.0
Newspaper	28	25.9
Continuing Medical Education (CME)	25	23.1
Seminar/Conference	23	21.3

Table 3: Resident doctors' knowledge of periodontal disease

Variables	n (109)	%
Understanding of the term " Periodontal disease"		
Same as gum disease*	75	68.8
Same as tooth decay	22	20.2
Same as oral cancer	0	0.0
Didn't know	12	11.0
Primary cause of Periodontal disease		
Dental plaque*	58	53.2
Sweets/sugary foods	28	25.7
Vitamin C deficiency	4	3.7
Don't know	19	17.4
Earliest sign of periodontal disease		
Swollen gum	46	42.2
Bleeding gum*	12	11.0
Bad breath	28	25.7
Holes in the teeth	12	11.0
Didn't know	11	10.1
Best method for preventing periodontal disease		
Daily Toothbrushing, fluoride paste, dental flossing*	62	56.9
Daily Toothbrushing, fluoride paste, mouthrinses	40	36.7
Daily Toothbrushing, any toothpaste, mouthrinses	4	3.7
Didn't know	3	2.8

* Correct response

Table 4: Resident doctors' knowledge of association between systemic diseases/ conditions and periodontal disease

Systemic condition	Yes n (%)	No n (%)	Not sure n (%)	Total n (%)
Smoking	100 (91.7)	1 (0.9)	8 (7.3)	109 (100)
HIV/AIDS	87 (79.8)	6 (5.5)	16 (14.7)	109 (100)
Diabetes mellitus	85 (77.9)	5 (4.6)	19 (17.4)	109 (100)
Leukemia	68 (62.4)	7 (6.4)	34 (31.2)	109 (100)
Stress	59 (54.1)	11 (10.1)	39 (35.8)	109 (100)
Chronic Kidney Disease (CKD)	56 (51.4)	10 (9.2)	43 (39.4)	109 (100)
Downs syndrome	40 (37.0)	9 (8.3)	59 (54.6)	109 (100)
Pregnancy	38 (34.9)	19 (17.4)	52 (47.7)	109 (100)
Osteoporosis	33 (30.6)	11 (10.2)	64 (59.3)	109 (100)
Erectile dysfunction	18 (16.5)	32 (29.4)	59 (54.1)	109 (100)
Rheumatoid arthritis	10 (9.2)	40 (36.7)	59 (54.1)	109 (100)

Table 5: Resident doctors' knowledge of periodontal disease as a risk factor for systemic diseases

Systemic condition	Yes n (%)	No n (%)	Not sure n (%)	Total n (%)
Hospital Acquired pneumonia	58 (53.2)	19 (17.4)	32 (29.4)	109 (100)
Coronary Heart disease	50 (45.9)	23 (21.1)	36 (33.0)	109 (100)
Stroke	47 (43.1)	22 (20.2)	40 (35.7)	109 (100)
Peripheral arterial disease	31 (28.4)	26 (23.9)	52 (47.7)	109 (100)
Diabetes Mellitus/Glycemic control	15 (13.7)	3 (0.0)	91 (86.3)	109 (100)
Preterm/low birth weight babies	12 (11.0)	28 (25.7)	69 (63.3)	109 (100)

Table 6: Resident doctors' knowledge of medications associated with periodontal disease

Medication	Yes n (%)	No n (%)	Not sure n (%)	Total n (%)
Phenytoin	76 (69.7)	3 (2.8)	30 (27.5)	109 (100)
Cyclosporin	48 (44.0)	4 (3.7)	57 (52.3)	109 (100)
Oral contraceptives	29 (26.6)	12 (11.0)	68 (62.4)	109 (100)
Nifedipine	18 (16.5)	22 (20.2)	69 (63.3)	109 (100)

Table 7: Attitude of Resident doctors towards their patients' periodontal health

Attitude	Strongly agree n (%)	Agree n (%)	Neutral n (%)	Disagree n (%)	Strongly Disagree n (%)	Non-response n (%)
Importance of assessing their patients regularly for periodontal disease	55 (50.5)	44 (40.4)	7 (6.4)	3 (2.8)	0 (0)	0 (0)
Referring their patients for routine dental check up	47 (43.1)	41 (37.6)	15 (13.8)	5 (4.6)	0 (0)	1 (0.9)
Referring their patients for dental care only when they had complaints	7 (6.4)	18 (16.5)	12 (11)	50 (45.9)	19 (17.4)	3 (2.8)
Referring their patients only on their request	1 (0.9)	16 (14.7)	12 (11	49 (45)	26 (23.9)	5 (4.6)

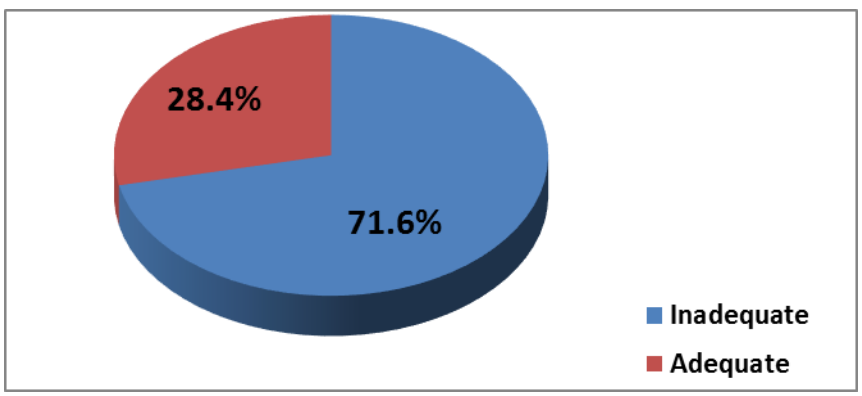

Figure A: Resident doctors' knowledge of periodontal disease

KNOWLEDGE OF PERIODONTAL DISEASES AND ORAL HYGIENE PRACTICES AMONG ADMINISTRATIVE STAFF IN LAGOS, NIGERIA

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ABSTRACT

Background: Periodontal (Gum) diseases are highly prevalent and the major cause of tooth loss among adults, hence of public health importance. Their prevention depends to a large extent on oral health knowledge, attitude, and practices. In Nigeria, there is low awareness of oral health issues and poor access to oral health facilities. There are limited studies on the knowledge and practices of Administrative staff in hospital settings about periodontal diseases. **Objectives:** To determine the knowledge of periodontal disease (PD) and oral hygiene practices of administrative workers within a hospital setting. **Methods:** Using a cross-sectional study design, self-administered, semi-structured, questionnaires were distributed to 329 administrative staff at the Lagos University Teaching Hospital. The questionnaire had sections on socio-demography, knowledge of PD and oral hygiene practices. Data was analyzed using Epi info version 3.5.4 software package. Chi-square and Anova tests were used to test associations. Level of significance was set at $p < 0.05$. **Results:** A total of 284 (86.3%) administrative workers filled the questionnaires correctly. Mean age was 41.5 ± 8.9 years, males representing 53.9%. Over 60% claimed to be aware of PD. Overall, only 29.8% had adequate level of knowledge of PD. This was significantly associated with age, education and ethnicity ($p<0.05$). Only 9.9% used the recommended dental floss for interdental cleaning. Majority (83.8%) attended the dental clinic for pre-employment screening. Less than half (49.6%) had cleaned their teeth professionally. **Conclusions:** The knowledge of PD among the Administrative staff was inadequate. Their dental attendance was mainly due to the hospital policy of pre-employment screening.

Keywords: *Knowledge, Oral hygiene, Practices, Periodontal disease, Administrative Staff.*

INTRODUCTION

Periodontal (gum) diseases are highly prevalent diseases and are the second major dental disease groups affecting mankind globally. Being the leading cause of tooth loss among adults in both developed and developing countries,[1-3] they are of public health importance.[2-4] Periodontal diseases are inflammatory in nature and mediated by bacteria within plaque biofilms. The major forms are gingivitis and periodontitis. Gingivitis can be readily reversed by effective plaque control measures such as daily tooth brushing and the use of interdental cleaning aids such as dental floss.[5-8] Although, periodontal diseases are largely preventable, this depends largely on individuals' oral health knowledge, attitude, practices, and access to dental care.[9-11-14]

In developing countries such as Nigeria, factors that hinder good oral health care delivery are low awareness level and poor access to oral health facilities.[15-18] The failure to access oral health care has been attributed to a lack of perceived need for dental treatment.[11]Oral health care facilities are located within medical service complexes in most government owned health institutions in Nigeria.[15] The closer proximity of hospital workers to these dental health facilities

is should encourage a higher level of awareness of periodontal disease and better geographic access to these health facilities.

Health workers especially administrative workers represent an important segment of the hospital workforce and merit proper attention given their key roles in sustaining the financial health of the hospital and in fulfilling management functions efficiently to support consistent, high-quality care.[19] They could also serve as positive change agents for oral health promotion to their families and by extension the general population. In some Tertiary health institutions in Nigeria, hospital workers are mandated to undergo dental screening as part of their pre-employment medical examination. A dearth of information however exists on the knowledge and oral hygiene practices of these workers in Nigeria. The objectives of this study were therefore to determine the level of awareness and knowledge of periodontal disease and the oral hygiene practices of administrative workers within a hospital setting in Nigeria.

METHODS

Study design

This was a cross sectional study of Administrative workers at the Lagos University Teaching Hospital, Idi-Araba Lagos and was part of a larger study. The inclusion criteria were those who had worked for at least one year within the hospital.

Data Collection

Self-administered, semi-structured, pre-tested questionnaires were distributed to all the Administrative workers who consented to participate in the study in July 2013. The questionnaire had 3 sections.

Section A was used to obtain information on socio-demography of the respondents; Section B assessed their awareness and knowledge of periodontal disease. In this section, 4 questions were used to assess their level of knowledge, each having one correct answer. A correct response was scored 1, wrong response scored 0. The scores ranged from 0-4. Their level of knowledge was based on scores 0-2 (inadequate) and 3-4 (adequate).

Section C assessed their oral self-care practices and pattern of dental clinic attendance.

Approval for the study was sought and obtained from the Research and Ethics Committee of the Lagos University Teaching Hospital before commencing the study. Written informed consent was obtained from each of the respondents. Collected data were analyzed using the EPI INFO statistical software package version 3.5.4. Categorical variables were reported as frequencies and percentages and presented as tables and figures. Continuous variables were reported as Mean ± Standard deviation (SD). Pearson Chi-square test of association was used to determine significant statistical associations for categorical variables. The level of statistical significance was set at p value < 0.05.

RESULTS

Two hundred and eighty four (86.3%) of the 329 respondents correctly filled the questionnaires. There were 53.9% males with an overall mean age of 41.5 years. The respondents were from 23 departments within the hospital, thus a wide representation. Table 1 summarizes the socio-demographic characteristics of the respondents. Of the respondents, 60.2% (n=171) of the respondents claimed to be aware of gum disease. See Figure A. Data on the 171 respondents was subsequently analyzed to determine their level of knowledge. Of these, 38.6% reported their main source of information as the dental clinic (Figure B). Their responses to questions assessing their knowledge of periodontal disease are shown in Table 2.

Overall, only 29.8% had adequate level of knowledge of gum disease (Table 3). This knowledge was significantly associated with age (p= 0.031), education (p=0.005) and ethnicity (p=0.027) (See Table 4). Their oral self-care practices are shown in Table 5. Table 6 shows their dental

attendance pattern. Majority (89.1%) had visited a dentist previously, 74.6% being for pre-employment dental screening. Most (80.6%) attended only when they had a dental problem. Less than half (49.6%) had undergone professional cleaning of their teeth. There was no significant association between the respondents' level of knowledge of gum disease and their oral hygiene practices such as teeth cleaning, use of dental floss and dental clinic attendance ($p>0.05$).

DISCUSSION

Most oral-biofilm-induced periodontal diseases are chronic in nature and advance slowly, hence may remain unnoticed by people until complications occur. It is imperative therefore for people to have a high level of awareness and knowledge of this disease. This study revealed a fairly high level of awareness of periodontal disease (60.2%) among the hospital workers. This stems from the fact that majority of the respondents had been opportune to attend the dental clinic within the hospital premises particularly during their pre-employment screening and may have received some form of oral health education during this period.
This is far greater than the 7% reported from another Nigerian study among an adult diabetic population.[20]

Their most common source of information about periodontal disease was the dental clinic which is comparable with other studies.[21,22] This may have arisen from the fact that dentists and other dental professionals are usually the trail blazers in most oral health campaigns in Nigeria. Food debris and not plaque was erroneously chosen by many workers as the main cause of gum disease. Dental plaque unlike food debris cannot be rinsed off by water or mouth rinse but must be removed by applying mechanical force such as tooth brushing. This finding is in accordance with other studies in China and India.[14,23] This is however in wide contrast to the 60.5% reported in Belarus (Eastern Europe).[24] Less than half of the respondents correctly identified gum bleeding as the most common sign of periodontal disease. This is a huge contrast to the 94% reported among young adults in Saudi Arabia.[25] This difference may be explained by the fact that the young individuals were studying in the University at the time, which could have encouraged them to seek more knowledge. A positive observation in this study was the large number of respondents who correctly affirmed a relationship between gum disease and general health. This is encouraging in view of the recent studies linking periodontal disease with some systemic diseases such as diabetes.[26]

The overall level of knowledge of gum disease was inadequate among the administrative workers (29.8%). This is similar to the 27.2% reported in another Nigerian study.[27] This may be attributed to their limited exposure to periodic oral health education programs within the hospital or lack of interest in hospital organized seminars. Workers that had higher knowledge of periodontal disease were found to be those in younger age groups, those with higher level of education and from minority ethnic groups. This is not surprising and highlights the importance of formal education in enhancing oral health knowledge[27,29-31] which can influence oral health behavior.[28] It is also very likely for respondents with tertiary education to have better access to appropriate sources of information on periodontal disease such as the Internet. A relationship between the ethnicity and level of dental health knowledge has also been reported in other studies.[30,32-34]

The importance of good oral hygiene practice in the maintenance of oral health cannot be overemphasized. Most of the respondents in this study (86.3%) used tooth brush and tooth paste which is the most ideal method of preventing gum disease. Others used chewing stick. Similar findings have been reported.[35,36] The use of chewing sticks is also an acceptable method of oral hygiene practice which has been advocated among the populace particularly in developing

countries because of its affordability, wide availability, antibacterial and anti-plaque properties.[17,37]

Despite the importance of the recommended twice daily cleaning to periodontal health, 43.3% in the present study practiced this. This is similar to the 43.2% and 47.5% reported in studies in Nigeria and Saudi Arabia respectively.[36,38] Our study does contrast with the 78% reported among Virginia residents in the United States.[39] More than two thirds (68.3%) of the respondents used soft-medium textured toothbrush which is preferred and recommended.[5] Although, there are limited studies on the factors influencing the choice of toothbrush selection and effect of texture on tooth tissues, soft-medium brushes are less likely to cause damage to the gums compared to hard bristle brushes.

Interdental cleaning aids such as dental floss was used by only 9.9% of the respondents. This contrasts with the 28% reported in the United States.[39] Most workers indulged in the use of potentially traumatic interdental cleaning aids to the gum such toothpicks, pins, and broomsticks. Probable reasons for this is the fact that habitually, people are used to toothpicks which are more readily available in markets, shops and are circulated in social gatherings in this environment. There is however a need to promote the use of dental floss for optimal periodontal health.

In the present study, 89.1% had visited the dentist before. This is more likely the result of the hospital policy of pre-employment screening for workers which is commendable. Only 48.2% had visited the dental clinic subsequently. This could imply the workers' poor attitude and motivation for preventive dental visits. This may be caused by the lack of perceived dental problem by the respondents as most of them had worked for an average of 13 years in the hospital. Over 80% visited the dental clinic only when they had a dental problem. Only 19.8% actually visited in the previous year, a finding corroborated in another study.[40] A recent study also reported dental problem as the most common reason for dental attendance among administrative workers within a hospital setting in Lagos, Nigeria.[41] This is in contrast with the 63% reported among United States citizens in Virginia whose reason for visiting was preventive rather than curative.[39] Researchers have highlighted the poor health seeking habits of Nigerians which is mainly for emergency relief of pain through tooth extractions. In addition, others have identified negative attitudes to oral health and out-of pocket payment for dental care as probable reasons.[17,22,40,42] Ignorance about the existing National Health Insurance Scheme and the non-inclusion of preventive dental care such as professional scaling and polishing in the scheme may be other reasons preventing these workers from seeking preventive dental care.

CONCLUSION

The knowledge of periodontal disease among the Administrative staff was inadequate. Their dental attendance practice was poor as majority visited the dentist because of the mandatory hospital policy of pre-employment dental screening and for a dental problem.

RECOMMENDATIONS

Periodic oral health education should be given with emphasis on education, preventive dental visits among administrative staff within hospital settings. Pre-employment dental screening for newly employed administrative workers is a good policy which should be maintained and periodic.

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Table 1: Distribution of respondents by their socio-demographic characteristics

Variable	n (284)	%
Age group (years)		
20-29	31	10.9
30-39	94	33.1
40-49	101	35.6
≥ 50	58	20.4
Mean age: 41.5 ± 8.9		
Gender		
Male	153	53.9
Female	131	46.1
Marital Status		
Single	52	18.3
Married	220	77.5
Widowed	12	4.2
Ethnicity		
Yoruba	209	73.6
Igbo	44	15.5
Others (Edo, Ibibio, Hausa)	31	10.9
Level of Education		
Secondary	87	36.6
Tertiary	197	69.4
Grade level		
Junior staff	90	31.7
Senior staff (Intermediate)	171	60.2
Senior staff	23	8.1
Duration of work (years)		
≤10	131	46.1
11-20	69	24.3
>20	84	29.6
Median duration of work: 13 years		

Table 2: Distribution of respondents according to knowledge of periodontal disease

Variable	n (171)	%
Main cause of gum disease		
Food debris	104	60.8
Poor nutrition	27	15.8
Dental plaque*	25	14.6
Didn't know	6	3.5
Calculus	5	2.9
Inherited from parents	4	2.3
Most common sign of gum disease		
Bleeding gum*	81	47.3
Swollen gum	71	41.5
Bad breath	13	7.6
Didn't know	5	2.9
Pain	1	0.6
Best method for preventing gum disease		
Cleaning with toothbrush and paste*	117	68.4
Rinsing with mouthwash	28	16.4
Rinsing with water	24	14.0
Didn't know	2	1.2
Relationship between gum disease and general health		
Yes*	126	73.7
No	35	20.5
Didn't know	10	5.8

* Correct response

Table 3: Distribution of respondents by their level of knowledge of periodontal disease

Level of Knowledge	Frequency (n)	Percent (%)
Inadequate	120	70.2
Adequate	51	29.8
Total	**171**	**100.0**

Table 4: Association between socio-demographic characteristics of respondents and level of knowledge of periodontal disease

Variable	No. of subjects (n=171)	Mean knowledge score	(±SD)	P value
Age (years)				
20-29	17	2.2	1.0	0.031*
30-39	67	2.1	0.8	
40-49	54	2.1	0.8	
≥50	33	1.6	1.0	
Gender				
Male	85	2.1	0.9	0.073
Female	86	1.9	0.9	
Educational level				
Secondary	38	1.7	0.9	0.005*
Tertiary	133	2.1	0.9	
Marital status				
Single	30	2.2	1.0	0.069
Married	133	2.0	0.9	
Widow	8	1.4	1.1	
Ethnicity				
Yoruba	130	2.9	0.9	0.027*
Igbo	23	3.4	0.7	
Others	18	3.3	1.0	
Work category				
Junior staff	53	2.0	0.9	0.445
Senior staff (Intermediate)	98	2.1	0.9	
Senior staff	15	1.8	1.2	
Duration of work (years)				
≤ 10	84	2.1	0.9	0.276
11-20	41	2.2	0.8	
> 20	46	1.9	1.0	

***Statistically significant**

Table 5: Distribution of respondents according to their oral hygiene self-care practices

Variable	n (284)	%
Frequency of daily cleaning of teeth		
Once	161	56.7
Twice*	116	40.8
More than twice*	7	2.5
Tooth cleaning aid used		
Toothbrush & paste*	245	86.3
Both Toothbrush/paste & Chewing Stick*	35	12.3
Chewing stick	4	1.4
Type of toothbrush used		
Soft*	82	29.2
Medium*	110	39.1
Hard	86	30.6
Didn't know	3	1.1
Frequency of changing toothbrush		
1-3 months*	155	54.6
4-6 months	100	35.2
More than 7 months	25	8.8
Not applicable	4	1.4
Interdental tooth cleaning aid most often used.		
Dental floss*	28	9.9
Toothpick	210	74.0
Others (pin, knife, broomstick, thread, fingernails)	14	4.9
None	2	0.7

***Recommended practice**

Table 6: Distribution of respondents according to their dental attendance practice

Variable	n	%
History of previous dental visit (n=284)		
Yes*	253	89.1
No	31	10.9
Frequency of dental visits (n=253)		
Once in 6 months*	9	3.6
Once in 6-12 months*	30	11.8
Occasionally	9	3.6
When I have a dental problem	204	80.6
When I have the time	1	0.4
Last dental visit (n=253)		
Less than a year ago*	50	19.8
More than a year ago	203	80.2
Reason for last dental visit (n=253)		
Pre-employment screening	58	22.9
Dental problem	116	45.8
Dental check-up*	79	31.2
Pre-employment dental screening (n=284)		
Yes	212	74.6
No	72	25.4
Visited the LUTH dental clinic after pre-employment dental check-up (n=284)		
Yes*	137	48.2
No	147	51.8
Professional cleaning of teeth at the dental clinic (n=284)	141	49.6
Yes*	143	50.4
No		
Last time teeth was cleaned at the dental clinic (n=141)		
Less than 6 months ago*	20	14.2
More than 6 months ago	121	85.8

*** Recommended practice**

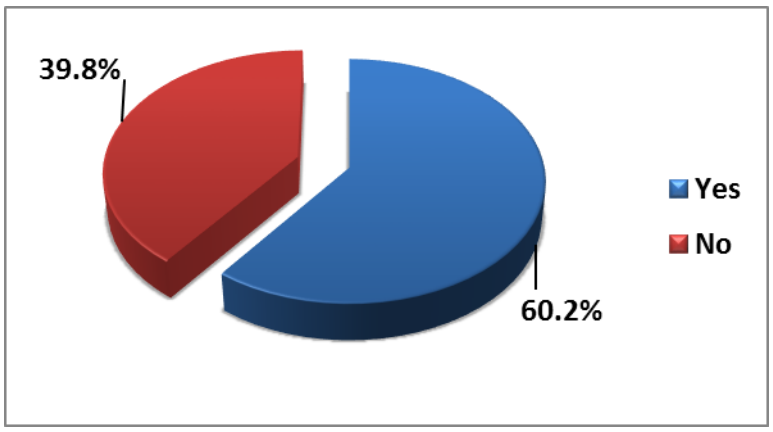

Figure A: Distribution of respondents by their awareness of periodontal disease

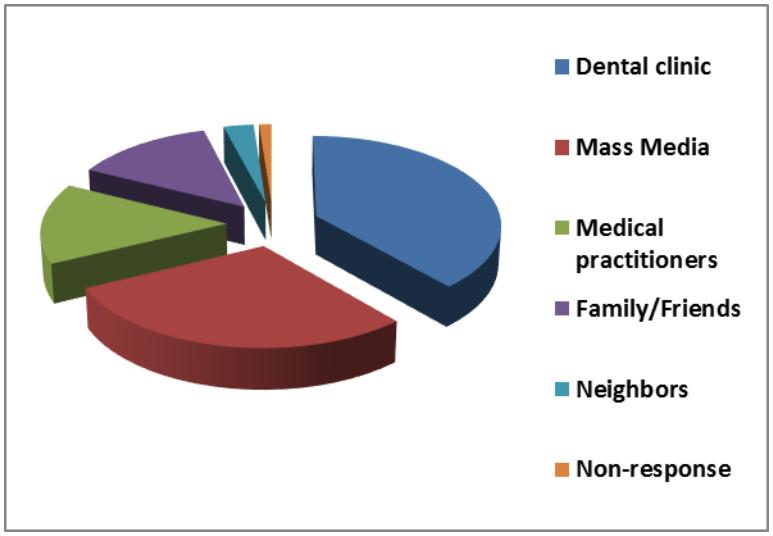

Figure B: Source of Information about oral health

EARLY CHILDHOOD CARIES AND ITS ASSOCIATION WITH BREASTFEEDING AND BOTTLE FEEDING AMONG PRESCHOOL CHILDREN

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ABSTRACT
Background: Concerns have been raised that breastfeeding and bottle feeding practices may increase the risk of early childhood caries (ECC). **Objectives:** The aim of this study was to assess the potential association of breastfeeding and bottle-feeding with the risk for early childhood caries among preschool children. **Methods:** Information about oral health, infant feeding and other child and family characteristics among children aged 6 -71 months (N=302) referred to outpatient paediatric clinics in Lagos were obtained through a structured questionnaire from their mothers/caregivers. The status of dental caries was recorded according to the World Health Organization (WHO) criteria. The data was analysed using Statistical Package for Social Sciences (SPSS) version 17. Statistical analyses of association of ECC with various categorical variables were performed using chi-square. Probability value $p \leq 0.05$ were considered statistically significant. **Results:** The overall caries prevalence of 302 children examined in this study was 21.2%. There was a statistically significant increase in the prevalence of ECC in children who were breastfed greater than 12 months of age (P=0.0002) and children who were bottle-fed at night (0.0001), however, the association between ECC and 'on-demand' and nocturnal breastfeeding were not statistically significant ($p \geq 0.05$). **Conclusion:** It was concluded that breastfeeding beyond 12 months of age, nocturnal bottle-feeding, are risk factors for ECC. Oral health promotion programmes with proper infant feeding practices should be directed towards mothers, nurses, paediatricians and primary health care workers.

Keywords: *Early Childhood, Caries, Breastfeeding, Bottle-feeding Lagos.*

INTRODUCTION

Early Childhood Caries (ECC) was formerly known as baby bottle tooth decay, nursing caries, nursing bottle syndrome, nursing caries, bottle mouth caries and milk bottle syndrome.[1-3] ECC is defined as the presence of one or more decayed, missing (due to caries), or filled primary tooth surface (dmfs) in any primary tooth in a child ≤ 71 months of age. Severe ECC is the presence of any sign of smooth surface caries in children < 3 years of age; ≥1 cavitated, missing (due to caries), or filled smooth surface in maxillary anterior teeth from ages 3 through 5 years; or the presence of ≥1 decayed, missing (due to caries), or filled primary tooth surfaces of ≥4 at age 3 years, ≥5 at 4 years, or ≥6 at age 5 years.[4] Dental caries comprise the single most chronic disease affecting children globally with the largest unmet health needs, if left untreated can lead to significant acute and chronic conditions, bacteraemia, early loss of tooth, malocclusion in the permanent dentition, high cost of treatment, low self esteem and failure to thrive.[1,5,6] Infant feeding habits such as at-will breastfeeding, prolonged and nocturnal breastfeeding, prolonged day time and nocturnal use of baby bottle that contain fermentable liquids, continued use of sweetened pacifiers and diet are some of the common risk factors for ECC.[7] The American Academy of Pediatrics identifies human milk as the ideal nutrient for infants on the basis of the extensive scientific evidence demonstrating that breastfeeding and the use of human milk will provide multiple health-related advantages to infants, mothers and society.[8] Breastfeeding is recommended by paediatricians and other healthcare professionals to be continued for at least the first year of life and beyond, as long as mutually desired by mother and child.[9] Prolonged, unrestricted and night time breastfeeding, however have been reported to be potential risk factors

for the development of ECC.[3,10-12]The purpose of this study is to determine the association between breastfeeding and bottle-feeding practices among preschool children referred to paediatric out-patient clinics at the Lagos university teaching Hospital (LUTH).

METHODS

Ethics

Approval for research was obtained from the Health Research and Ethics Committee of the Lagos University Teaching Hospital and informed consent was obtained from mothers before each child was enrolled.

Sampling

The study was carried out among children aged 6 to 71months selected from patients referred to four different paediatric outpatient clinics in LUTH.

Proportionate selection sampling was used to recruit children from the four paediatric outpatient clinics according to the average number of children attending the clinic in a month. At each clinic every alternate child who met the inclusion criteria was selected from each clinic until the desired number of subjects in each clinic and minimum sample size was attained.

Infant Breastfeeding Data

A structured questionnaire was used. Information about infant feeding was obtained from parents/caregivers of children during an in-person interview. The questionnaire consisted of demographic information on the child, feeding practices, which included the type of infant feeding method (breastfeeding, bottle feeding or mixed), on-demand and nocturnal breastfeeding; nocturnal bottle-feeding; and duration of breast and bottle feeding.

For the purpose of this study the socioeconomic status (SES) was based on a composite of two indices: mother's education and father's occupation. This allocated each child to a social class I to V, social class V being at the bottom of the table.

Clinical Examination

The chief investigator and two other paediatric dentists were calibrated to carry out the clinical examination of the children according to the WHO oral health survey methods. The examiners were calibrated before the survey. Kappa scores higher than 0.9 were attained for both inter and intra-examiner calibration exercises for identifying cavitated and non- cavitated carious lesions indicating high reliability between investigators.

The children were examined by using disposable explorer, dental mouth mirrors and flashlights. During the examination, the older children were seated on a chair and infants were examined with assistance of their mothers', by means of the "knee-to-knee" technique. Gauze pads were used to clean and dry teeth surfaces before examination.

Children having one or more decayed (noncavitated or cavitated), missing (due to caries), or filled tooth surfaces (dmfs) in any primary tooth up to 71 months of age or younger were considered to have ECC. Children from ages 3 through 5, with one or more cavitated, missing (due to caries) or filled smooth surfaces in primary maxillary anterior teeth or decayed, missing, or filled score of ≥ 4 (age3), or ≥ 5 (age 4), or ≥ 6 (age 5) surfaces were considered to have S-ECC. Children with untreated caries were referred to the Department of Child Dental Health LUTH.

Data Analysis

The data was analysed using Statistical Package for Social Sciences (SPSS) version 17. Data analysis included descriptive statistics, comparisons of means and test of association. Statistical

analyses of association of ECC with various categorical variables were performed using Chi-square and Fisher's exact test. Probability value $p \leq 0.05$ were considered statistically significant.

RESULTS

A total number of 302 children were examined in the study. The age of the children ranged from 6 to 70 months with a mean age of 36.9 months (+/- 19.7 months). There were 158 (52.5%) girls and 144 (47.7%) boys. The highest distribution of children was in the age group 36 to 41 months. Most of the respondents were Yoruba 151 (50%), followed by Igbo 102 (33.8%). Majority of the children 118 (39.1%) were from the high socio-economic class I. (Table 1)

The overall caries prevalence of 302 children examined in this study was 21.2%. One hundred and thirty four (44.4%) children were exclusively breast fed, only 6(1.99%) were exclusively bottle-fed and the rest 162 (53.6%) were both breast and bottle fed. There was higher caries prevalence among children who were exclusively bottle-fed (33.3%). There was no statistically significant difference in the three methods of infant feeding with regards to the prevalence of dental caries among the children, (P=0.089) [Table 2]

The prevalence of ECC increased significantly with long duration of breastfeeding. Among those that were breastfed for 12 months or less, a caries prevalence of 7- 25% was recorded while those that were breastfed for more than 12 months had a prevalence of 57%. The longer the duration of breastfeeding, the higher the prevalence of ECC. There was a statistically significant association between the duration of breastfeeding and the prevalence of dental caries (P= 0.0002) [Table 3]]

Out of 257 (86.8%) children who were breastfed on demand, 54 (21%) had ECC while 39 (13.2%) children who were not breastfed on demand 8 (20.5%) had ECC. Although there was a marginal increase in the prevalence of ECC in those that were breastfed on demand, this relationship was not statistically significant (P= 0.943). The prevalence of caries was higher (21.2%) in those children who were breastfed at night than those who were not (12.5%) but there was no statistically significant difference in the two groups (P=0.4) [Table 4]

More than half 100(59.5%) of the children were bottled-fed for less than 12 months. Children that were bottle-fed more than 2 years had higher prevalence (50%) of ECC. There was no statistically significant association between the duration of bottle-feeding and the development of ECC P= 0.668. Out of the 55 children who were bottle-fed at night, a significantly higher percentage (51%) had ECC compared to those who were not bottle-fed at night (13%). There was a statistically significant association between children who were bottle-fed at night and those who were not and the occurrence of ECC (P= 0.0001) [Table 5].

DISCUSSION

The idea that breastfeeding- especially on demand at night can lead to an increased risk for ECC has concerned the dental community since it was first raised in the literature.[13] Kotlow presented case reports of clinical observations suggesting that breastfeeding on demand may be associated with ECC.[13]

The present study has identified several characteristics of breastfeeding and bottle-feeding habits and ECC. Nocturnal and at-will breastfeeding were not associated with ECC but breastfeeding in children greater than 12 months was strongly associated with ECC (P=0.0002). This finding is similar to that by Azevedo et al and Shantinath et al.[3,14] Shantinath et al showed that the average age of weaning was 6 months earlier for caries-free group than for caries group.[14] In Azevedo's study, breastfeeding in children older than 12 months was strongly associated with S-ECC.[3] Yonezu et al in their study reported that breastfeeding at 18months was significantly associated with the higher prevalence of caries and higher number of decayed, missing and filled teeth.[15] Other studies have reported that there is no association between breastfeeding and ECC.[16-18] In

the present study although the prevalence of ECC was lowest in those who were exclusively breastfed and highest among those who were exclusively bottle-fed, the differences in caries levels in relation to type of feeding was not statistically significant this is similar to other reports.[19] Some authors have reported that children who were never breastfed or those that were wholly bottle-fed have a higher risk for ECC when compared to breastfed babies.[10,20,21] Breastfeeding provides the perfect nutrition for infants, and there are a number of health benefits to the breastfed child, including reduced risk for gastrointestinal and respiratory infections. However, frequent and prolonged contact of enamel with human has been shown to result in acidogenic conditions and softening of enamel.[22] The American Dental Association's (ADA) statement on ECC states that 'unrestricted, at-will nocturnal breastfeeding after the eruption of the child's first tooth can lead to an increased risk of caries'.[23] The American Academy of Pediatric Dentistry (AAPD) Policy on early Childhood Caries recommends that 'Ad libitum breast-feeding should be avoided after the primary tooth begins to erupt and other dietary carbohydrates are introduced'.[24] In the present study bottle feeding at night was a clear determinant for ECC, there was a significantly higher prevalence of ECC in children who were bottle-fed at night than those who were not . This report agrees with other studies that examined bottle-feeding in detail that duration of bottle feeding particularly at night is the most important determinant for ECC development rather than bottle-feeding itself.[18,25] Feeding during the night can lead to prolonged exposure to fermentable carbohydrates and also salivary flow and function is reduced during sleep creating dentally harmful environment.[18] The relationship between breastfeeding and ECC could not be established in many studies. The period of breastfeeding is also controversial.

Based on the finding from the present study, it is suggested that infants should not be put to sleep with a bottle filled with milk or liquids containing sugars, ad libitum breastfeeding should be avoided after the first primary tooth begins to erupt and other dietary carbohydrates are introduced, parents should be encouraged to have infants drink from a cup as they approach their first birthday. Infants should be weaned from the bottle between 12 to 18 months of age in accordance with the statement AAPD.[24]
Result from the present study also suggests that there is greater risk of developing ECC as breastfeeding continues beyond the age of 12 months, we recommend that night time breastfeeding be avoided as it will be unrealistic to clean the baby's mouth after breastfeeding at night, also mothers who wish to continue breastfeeding children after the age of 12 months should make sure that child has adequate diet and good oral hygiene should be practiced.

CONCLUSION

The results from this study showed that breastfeeding beyond12 months of age and night-time bottle feeding are associated with early childhood caries. Oral health promotion programmes with proper infant feeding practices should be targeted at mothers, nurses, paediatricians and primary health care workers.

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Table 1: Distribution of respondents according to gender, ethnic group and socio-economic status

Characteristics	**Frequency**	**Percent**
Gender		
Male	144	47.7
Female	158	52.3
Total	**302**	**100.0**
Ethnic group		
Yoruba	151	50.0
Igbo	102	33.8
Hausa	18	6.0
Efik and Urhobo	31	10.3
Total	**302**	**100.0**
Socio-economic status (SES)		
I	118	39.1
II	56	18.5
III	80	26.5
IV	32	10.6
V	16	5.3
Total	**302**	**100.0**

Table 2: Association between infant feeding methods and ECC

Method of Feeding	**Caries free**		**Caries Affected**		**Total**		**P value**
	Freq	**%**	**Freq**	**%**	**Freq**	**%**	
Breast	113	84.3	21	15.7	134	44.37	0.089
Bottle	4	66.7	2	33.3	6	1.99	
Both	121	74.7	41	25.3	162	53.64	
Total	**238**	**78.8**	**64**	**21.2**	**302**	**100.0**	

*Fisher's exact test is significant at level ≤ 0.05
**Chi-square is significant at level ≤0.05

Table 3: Association between breastfeeding duration and ECC

BRF Duration	Caries free		Caries Affected		Total		P value
	Freq	**(%)**	**Freq**	**(%)**	Freq	(%)	
< 3 months	9	75.0	3	25.0	12	4.1	0.0002*
3-6 months	102	93.0	8	7.0	110	37.2	
7-12 months	92	90.0	10	10.0	102	34.5	
>12 months	31	43.0	41	57.0	72	24.3	
Total	**234**	**79.0**	**62**	**21.0**	**296**	**100.0**	

Table 4: Association between on-demand, nocturnal breastfeeding and ECC

Breast Feeding	Caries free		Caries Affected		Total		P value
	Freq	**(%)**	**Freq**	**(%)**	**Freq**	**(%)**	
On Demand							
Yes	203	79.0	54	21.0	257	86.8	0.943
No	31	79.5	8	20.5	39	13.2	
Total	234	79.1	62	20.9	296	100.0	
At night							
Yes	227	78.8	61	21.2	288	97.3	0.400
No	7	87.5	1	12.5	8	2.7	
Total	**234**	**79.1**	**62**	**20.9**	**296**	**100.0**	

Table 5: Association between duration of bottle-feeding, nocturnal bottle-feeding and ECC

	Caries free		Caries Affected		Total			P value
	Freq	**(%)**	**Freq**	**(%)**	**Freq**	**(%)**		
Duration								
<12	72	72.0	28	28.0	100	59.5		0.668
13 – 18	45	78.9	12	21.1	57	33.9		
19-24	7	77.8	2	22.2	9	5.4		
>24	1	50.0	1	50.0	2	1.2		
Total	125	74.4	43	25.6	168	100.0		
Sleep with bottle								
Yes	27	49.0	28	51.0	55	32.7	25.573	0.0001**
No	98	87.0	15	13.0	113	67.3		
Total	**125**	**74.0**	**43**	**26.0**	**168**	**100.0**		

ESTIMATION OF TOTAL CAROTENOIDS AND FREE RADICAL SCAVENGING ACTIVITY OF SELECTED VEGETABLES

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ABSTRACT
Vegetables are known for their rich carotenoid content primarily responsible for their antioxidant properties. Carotenoids are pigments found in plants and some animals responsible for colours ranging from yellow to red.
The aim of the study was to estimate the total carotenoids content (TCC) in four locally consumed Nigerian vegetables: *Telfairia occidentalis* Hook (Curcurbitaceae) leaves, *Carica papaya* Linn. (Caricaceae) leaves, *Capsicum annum* Linn. (Solanaceae) fruits and *Daucus carota* Linn. (Apiaceae) roots, compare their free radical scavenging activity (FRSA) and relate the total carotenoids in these vegetables to their respective free radical scavenging activities.
The vegetables were collected fresh, authenticated by taxonomists and museum specimens deposited at the herbarium. The fresh processed vegetables were homogenized with ethanol. Estimation of their TCC was done using UV spectrophotometry by obtaining the absorbances of a 1 in 25 dilution of each homogenate taken at 470 nm, 649 nm and 665 nm to estimate the concentrations (µg/mL) of Chlorophylls A and B. To obtain the TCC (µg/mL), a derived equation quoted in related studies was used to estimate it from the values of Chlorophylls A and B. FRSA was determined by measuring the decrease in the visible absorbance of Diphenylpicrylhydrazyl (DPPH) on addition of plant homogenates by taking the absorbance using UV spectrophotometry at 517 nm. The mean inhibitory concentration (IC_{50}) was determined graphically.
Results obtained for the TCC of the samples were in the order of *C. annum* > *C. papaya* > *T. occidentalis* > *D. carota* and for the FRSA (IC_{50}) of the samples in the order of *C. papaya* > *C. annum* > *T. occidentalis* > *D. carota*. *C. annum* had the highest TCC while *C. papaya* had the highest FRSA. The FRSA ranged from 1.65% to 71.12%.
The results showed a relationship between TCC and FRSA for all plants except *C. papaya* (Pawpaw) which had the highest FRSA but not the highest TCC. This may have been due to the presence of other antioxidants in *C. papaya* than in other vegetables. The results also indicated that the content of the vegetables mopped up free radicals to different extents. The results suggests that *C. papaya* leaves should be included in our diet because of its high antioxidant activity.

Keywords*: Vegetables, Carotenoids, Antioxidant, Pawpaw*

INTRODUCTION

The art of using our food as cure for bodily ailments is as old as mankind itself. Plant materials were among the treatments for diseases in the early days of man. The effects of plants materials could have been found through trial and error and may have led to wellness and if such plant is poisonous, death. Therefore, with the experience garnered by our early ancestors, edible plants were designated as food and medicine and poisonous plants were avoided. In early days of man, man was plagued with chronic diseases. Hence, it raised a serious concern among the great minds of those days. They called a meeting in 2nd century BC and came to a conclusion that "it is the wholesome use of food that promotes the health of a person and that which is unwholesome is the cause of disease". The implication of this statement boils down to the fact that the origin of the vast majority of our health problems is what we put in our bodies every day. Therefore, if we eat healthy, we would be healthy and healthy food intake can help fight diseases

(Caldecott, 2011). Thus, over a long period in human history, use of food has been advocated to fight diseases.

Free radicals have been implicated as agents that contribute to progression of chronic diseases. Free radicals can be defined as atoms or molecules containing one or more unpaired electrons in their orbitals. They are formed continuously in body cells as a consequence of both enzymatic and non-enzymatic reactions. It has been estimated that the average person has around 10000–20000 free radicals attacking each body cell each day (Pala and Gurkan, 2008). Some free radicals are good in that they enable the human body to fight inflammation, kill bacteria, and control the tone of smooth muscles, which regulate the working of internal organs and blood vessels. Conversely, increased or uncontrolled free radical activity might combine with other factors to cause some diseases such as neurodegenerative diseases, heart disease, cancers and so on. The balance between the production of free radicals and the antioxidant defences in the body has important health implications. Under the normal conditions the antioxidant defense system within the body can easily handle free radicals that are produced. If there are too many free radicals produced and too few antioxidants, this may cause chronic damage (Pala and Gurkan, 2008).

Therefore, to maintain the balance of free radicals and antioxidants, we must ensure that we take in enough food rich in antioxidants to make sure that this balance remains in our favour. Foods from plants and animals are very rich sources of these natural antioxidants (Rietjens *et al.*, 2002). Carotenoids are natural colourful plant pigments found in fruits and vegetables. Between 500 and 600 specific carotenoids have been identified of which only about 24 commonly occur in human foodstuff. The principal carotenoids of food are β-carotene, β-cryptoxanthin, Lycopene, Lutein and Violaxanthin. In local Nigerian diet, sources of carotenoids include Fluted gourd, *Telfairia occidentals* Hook leaves which has high content of β-carotene (Badifu *et al.*, 1995), Pawpaw or Papaya *Carica papaya* Linn. leaves (Maisarah *et al*., 2013), Red pepper *Capsicum annum* Linn. fruits (Southon and Faulks, 2003) and Carrot *Daucus carota* Linn. roots (Mech-Nowak *et al*., 2012).

Carotenoids carry out their antioxidant activity by free radical scavenging. Free radical scavenging is achieved by different mechanisms such as quenching of singlet oxygen, addition, electron transfer and hydrogen atom transfer (Jaswir *et al.*, 2011). Thus, they reduce the amount of free radicals in the cells and hence prevent instigation of illnesses. Knowledge exists that show that carotenoid-rich vegetables are invaluable in prevention of chronic illnesses.

METHODS

Collection And Identification Of Plant Materials Used: The plant materials were sourced from the market and their natural habitats in May 2013. The pawpaw leaves were collected from Ifako-Ijaiye area of Lagos state. The carrots were purchased from the vegetable garden market opposite Lagos University Teaching hospital, Idi-araba. The dried pepper fruits and fluted gourd leaves were purchased from the vegetable market at Mushin. The plants were identified and authenticated at the Department of Botany, University of Lagos. Museum specimens were kept at the herbarium.

Extraction Procedure: The method employed is a modified extraction process similar to that used by Dere *et al.* (1998). The carotenoids were obtained from the plant material in the fresh form for the leaves of the fluted gourd and pawpaw and root of carrots. Fresh leaves of fluted gourd and pawpaw and roots of carrots were washed and rinsed with water. The plant material was cut using a table knife into smaller sizes. Thereafter, 500 g of the fresh plant materials which were the fluted gourd leaves, carrots and Pawpaw were put into a Moulinex blender and homogenized with 500 mLs of Absolute Ethanol as the solvent. The dried pepper was also

reduced in size by dicing with a table knife and homogenized with the blender. For the dried pepper fruits, because of the nature of the material, 300 g of the plant required 400 mLs of Absolute ethanol to completely homogenize it. The blended plant material was then filtered using a glass funnel with muslin cloth then cotton wool to obtain the filtrate and stored in amber bottles in the refrigerator until used.

Estimation of Chlorophyll A, Chlorophyll B and Total Carotenoids: The extracts stored in the amber bottles were brought out from the refrigerator. Due to the sensitivity of the UV spectrophotometer, the extracts have to be well diluted until a clear solution was obtained. Finally, a 1 in 25 dilution was done for the stock solution to obtain a clear solution. 1 mL of the stock solution was made up to 5 mLs in a test tube with Absolute ethanol. This resulted in a 1 in 5 dilution. To obtain a 1 in 25 dilution, 1 mL of the first dilution was made up to 5 mLs in a test tube with Absolute ethanol. This procedure was done in triplicates for the different plant extracts. The equation proposed by Lichtenthaler and Wellburn (1983) for the determination of total carotenoids and chlorophylls a and b in extracts in different solvents including Absolute ethanol was employed. Absorbances of the diluted extracts already in triplicates were taken at 470 nm, 649 nm and 665 nm and values recorded. The concentrations (µg/ml) of the Chlorophyll a (C_a), Chlorophyll b (C_b) and Total carotenoids (C_{x+c}) were determined using the formulae stated below:

$C_a = 13.95\ A_{665} - 6.88A_{649}$

$C_b = 24.96A_{649} - 7.32A_{665}$

$C_{x+c} = 1000A_{470} - 2.05\ C_a - 114.8\ C_b$

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A_{470}, $A_{649}$and A_{665} represent the absorbance readings that were taken at 470 nm, 649 nm and 665 nm respectively.

The method used for extraction and spectrophotometric determination has been employed by Dere *et al.* (1998).

The result obtained was given as µg/mL. To calculate the amounts of pigments present per gram (µg/g), the result obtained was multiplied by 25 which is the dilution factor. This gives the amount of pigment per 1 g of plant material because:

500 g of plant material was homogenized with 500 mLs of solvent, therefore;

1 g of plant material is present in 1 mL of solvent.

However, for the dried plant material (Pepper):

300 g of plant material was homogenized with 400 mLs of solvent, therefore;

0.75 g of plant material is present in 1 mL of solvent

Therefore, amount of pigments per 0.75 g of plant material will be the result obtained multiplied by 25.

To obtain the amount of pigments (µg/g), the amount of pigment gotten from above was divided by 0.75.

Diphenylpicrylhydrazyl (Dpph) Free Radical Scavenging Activity: The method employed was a modification of the methods employed by Ayoola *et al*., (2008).

0.0079 g of DPPH was weighed on a chemical balance and transferred into a 200 mL volumetric flask. Sufficient amount of Absolute ethanol was then added and crystals dissolved in it to obtain the 1mM DPPH.

The stable 1,1-diphenyl-2-picryl-hydrazyl radical (DPPH) was used for a rapid determination of qualitative antioxidant activity by its free radical-scavenging activity of the extracts. Briefly, to a 2 mL ethanolic solution of extract of various concentrations at 0.2%, 0.4%, 0.6%, 0.8% and 1% of the stock of the raw homogenates (1 g/mL) of the fresh plant materials was added 1ml of 1mM DPPH. The pepper raw homogenate (0.75g/mL) was also adjusted to obtain 0.2%, 0.4%,

0.6%, 0.8% and 1%. A blank solution was prepared containing 2 mL of ethanol and 1 mL of 1mM DPPH.The experiments were carried out in triplicates. The test tubes were incubated for 15 minutes. Absolute ethanol was used to zero the spectrophotometer and the absorbance was read at 517nm. The free radical scavenging activity was calculated using the following formula.
% Inhibition of DPPH = $\{(A_B - A_A)/A_B\}$ x100 where A_B is the absorption of blank sample and A_A is the absorption of tested homogenate solution.. The results were expressed as percentage inhibition of DPPH.

Determination Of IC_{50} Values: Mean Inhibitory concentration (**IC_{50}**) value is the concentration that causes 50% inhibition of DPPH free radicals and is a parameter widely used to measure antioxidant/free radical scavenging power (Qian and Nihorimbere, 2004). It was obtained by using Graph Pad Prism Demo Software (Prism 6) using linear equation of the line. On a plot of the percentage inhibition against the concentration of the extract, a trend line equation: y = ax + b was determined. The values were calculated by transforming the equation above and the expression x (concentration) at which y-value (percentage DPPH inhibition) is 50% was accepted as unknown (Sierzant and Gabrielska, 2009). The values were computed and meaningful inferences made.

Statistical Analysis/Evaluation: The results of the concentration of the chlorophyll A, chlorophyll B and total carotenoids in the plant samples were subjected to statistical evaluation by obtaining the mean values, standard deviation and standard error of the means of each sample used.
The observations of chlorophyll A, chlorophyll B and total carotenoids were expressed as Mean ± Standard error of mean(SEM), n=3.
The observations of the percentage of inhibition of DPPH were also expressed as Mean ± Standard error of mean(SEM), n=3.
Graph Pad Prism Demo software (Prism 6) and Microsoft Excel were used to carry out the statistical analysis.
Tukey two-way ANOVA Test was also used to compare the differences in percentage of DPPH inhibition at different concentrations among the different plant extracts. The tests were done at 99% (Alpha=0.01) confidence intervals and meaningful inferences drawn from them.

RESULTS

Chlorophylls A & B Content And Total Carotenoid Content: Results of the Chlorophylls A & B and TCC are represented in Tables 1, 2 and 3 below.

DPPH Free Radical Scavenging Activity And IC_{50} Determination

The results of the DPPH scavenging activity are expressed in tables 4 and 5 below.

Statistical Comparison Of DPPH Inhibition Of Extracts: Statistical evaluation/ analysis of the different concentration of homogenates are expressed in table 6 below.

IC_{50}values Of Plant Extracts: IC_{50} value of each homogenate was also determined from linear plots as represented in Figure A below. The **IC_{50}** values of each homogenate are represented in table 7 below.

DISCUSSION

Estimation Of Total Carotenoid Content: This was compared to work done by several workers which showed the following total carotenoid contents as seen in table 8 below
Inspite of the differences observed in the total carotenoids, there seems to be an obvious trend in the carotenoid contents. The above table shows that Pepper fruits have more total carotenoids than Carrots as shown by the different studies above. This trend is consistent with the results of

this study that shows that Pepper fruits have the most amounts of total carotenoids and carrots have the least amount of total carotenoids exemplified by the order below.
Dried Pepper Fruits > Pawpaw leaves > Fluted gourd leaves > Carrots

DPPH Free Radical Scavenging Activity: Based on the results, the FRSA of the extracts was found to be (in descending order):
Pawpaw leaves > Dried Pepper fruits > Fluted Gourd leaves > Carrots
It was found that Pawpaw leaves have the highest FRSA of 71.12% at the highest concentration used almost 300% or triple of the activity of the next plant, the dried Pepper fruits which had 24.28% and more than triple of the activity of Fluted gourd leaves which had activity of 23.44% at the highest concentration used.
Carrots had the lowest FRSA of 4.50% at the highest concentration used in the study. This was far lower than that of the plant of the highest activity, Pawpaw leaves which had activity about more than 15 times than it.
This result shows that in this study Pawpaw leaves showed the highest anti-oxidant capacity and Carrots showed the lowest anti-oxidant capacity.

Relationship Between Carotenoid Content And FRSA: The results show a direct relationship between TCC and FRSA for two of the plants viz; Fluted gourd leaves and Carrot roots. However, there was an exception for Pawpaw leaves which did not have the highest TCC but had the highest FRSA. This can be assumed to be due to the fact that the antioxidant property of Pawpaw leaves was not due to just the presence of carotenoids alone but existence of other antioxidants.

Statistical Comparison Of DPPH Inhibition Of Extracts: The DPPH FRSA of the different plant extracts were compared with each other using the Tukey's Two way ANOVA analysis at 99% confidence interval, $p<0.01$.
After statistical analysis and evaluation, the following order was obtained based on their FRSA:
Pawpaw leaves > Dried Pepper fruits=Fluted gourd leaves > Carrots
FRSA of Pawpaw leaves was far superior to that of the other plants.

IC_{50} Values Of Plant Extracts
The extract with the lowest **IC_{50}** is said to have the strongest anti-oxidant capacity because a lower concentration is required to reduce the concentration of DPPH free radicals by 50%.
According to the results, Pawpaw leaves had the lowest value of **0.6057** making it the plant with the strongest anti-oxidant activity, this is followed by dried Pepper fruits with value of **1.9734** and Fluted gourd leaves with a value of **2.8805**. Carrots have the highest value of all the extracts with a value of **12.2325**.

The order based on the **IC_{50}**value from the highest to lowest is:
Carrots > Fluted gourd leaves > Dried Pepper Fruits > Pawpaw leaves.
Since the lowest value indicate higher activity, the order based on activity, will therefore be:
Pawpaw leaves > Dried Pepper fruits > Fluted Gourd leaves > Carrots.
Pawpaw leaves had the strongest anti-oxidant capacity and Carrots had the least anti-oxidant capacity.

CONCLUSION
Total carotenoid content of the four plants in the study showed that the dried *Capsicum annum* fruits had the highest carotenoid content and *Daucus carota* had the lowest carotenoid content.

All four plants showed free radical scavenging property. *Carica papaya* leaves had the best FRSA and *Daucus carota* had the least FRSA.

TCC is directly related to their FRSA. However, the plants that had higher FRSA in relation to their TCC may contain other antioxidants like phenols. In addition, it is suggested that Pawpaw leaves which is not part of the normal Nigerian diet should be included because it is a strong antioxidant.

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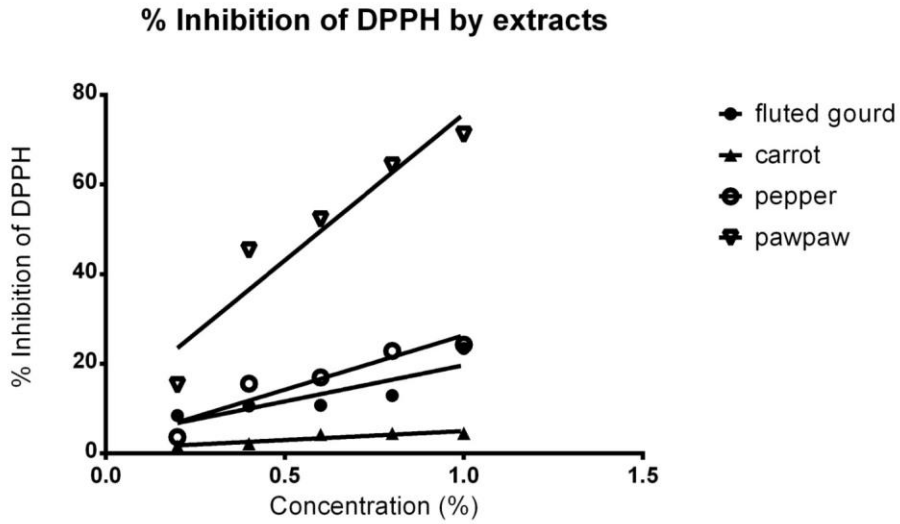

Figure (A): Determination Of IC_{50} Using Linear Plots

Equations Of The Linear Plots

$y = 16.12x + 3.581$, $R^2 = 0.7433$, Fluted gourd

$y = 4.005x + 1.009$, $R^2 = 0.8383$, Carrot

$y = 24.27x + 2.115$, $R^2 = 0.8828$, Pepper

$y = 65.31x + 10.44$, $R^2 = 0.9072$, Pawpaw

Table 1: Chlorophyll A, Chlorophyll B and Total Carotenoid Content

PLANTS	CHLOROPHYLL A (Ca)					CHLOROPHYLL B (Cb)					TOTAL CAROTENOIDS (Cx+c)				
	1ST	2ND	3RD	Mean	SEM	1ST	2ND	3RD	Mean	SEM	1ST	2ND	3RD	Mean	SEM
Daucus carota L.	0.4764	0.5195	0.4905	0.4955	0.0127	1.7783	2.0545	1.8136	1.8821	0.0868	0.1301	(0.0609)	0.0318	0.0337	0.0552
Capsicum annum L.	0.4437	0.3243	0.3115	0.3598	0.0421	0.7279	0.5984	0.8614	0.7292	0.0759	2.4307	3.0271	3.6877	3.0485	0.3630
Carica papaya L.	7.4255	4.2061	4.4394	5.3570	1.0364	1.7186	10.3680	4.6733	5.5866	2.5383	2.3571	(1.7668)	0.4588	0.3497	1.1917
Telfairia occidentalis Hook	3.1453	3.4845	3.9995	3.5431	0.2483	4.5197	3.7652	4.7974	4.3608	0.3084	(0.2706)	0.4637	0.4615	0.2182	0.2444

Table 2: Chlorophyll A & B Content

Plant	Chlorophyll A Content (µg/Ml) Of Extract	Chlorophyll A Presentµg /G of Plant Material	Chlorophyll B Content (µg/Ml) of Extract	Chlorophyll B Presentµg /G of Plant Material
***Daucus carota* Linn. (Apiaceae)**	0.4955±0.0127	12.3875	1.8821±0.0868	47.0525
***Capsicum annum* Linn. (Solanaceae)**	0.3598±0.0421	11.9933	0.7292±0.0759	24.3067
***Carica papaya* Linn. (Caricaceae)**	5.3570±1.0364	133.9250	5.5866±2.5383	139.6650
***Telfairia occidentalis*Hook (Cucurbitaceae)**	3.5431±0.2483	88.5775	4.3608±0.3084	109.0200

Table 3: Estimation of Total Carotenoids

Plant	Total Carotenoids (µg/mL) of extract	Carotenoids Present µg /g of plant material
***Daucus carota* Linn. (Apiaceae)**	0.0337±0.0552	0.8425
***Capsicum annum* Linn. (Solanaceae)**	3.0485±0.3630	101.6167
***Carica papaya* Linn. (Caricaceae)**	0.3497±1.1917	8.7425
***Telfairia occidentalis*Hook (Cucurbitaceae)**	0.2182±0.2444	5.4550

TABLE 4: PERCENTAGE INHIBITIONOF DPPH BY EXTRACTS

PLTS	0.2%			0.4%			0.6%			0.8%			1.0%		
	1ST	2ND	3RD	1ST	2ND	3RD	1ST	2ND	3RD	1ST	2ND	3RD	1ST	2ND	3RD
***Daucus carota* L.**	2.46 %	1.32 %	1.16 %	2.13 %	0.50 %	3.91 %	4.02 %	5.95 %	2.75 %	5.09 %	2.73 %	5.66 %	0.57 %	6.03 %	6.91 %
***Capsicum annum* L.**	0.00 %	10.66 %	0.35 %	17.12 %	18.71 %	10.88 %	17.62 %	16.00 %	17.30 %	27.74 %	26.96 %	13.94 %	23.16 %	24.75 %	24.92 %
***Carica papaya* L.**	26.84 %	5.70 %	13.27 %	51.03 %	39.17 %	45.69 %	56.34 %	50.74 %	49.59 %	63.42 %	62.40 %	66.83 %	75.37 %	66.61 %	71.39 %
***Telfairia occidentalis* Hook**	7.98 %	8.18 %	9.32 %	10.08 %	15.64 %	6.13 %	15.20 %	3.32 %	13.73 %	11.16 %	14.82 %	12.87 %	25.38 %	24.37 %	20.57 %

Table 5: DPPH free radical scavenging activity (% inhibition)

% INHIBITION	0.2	0.4	0.6	0.8	1.0
***Daucus carota* Linn. (Apiaceae)**	1.65±0.41	2.18±0.99	4.24±0.93	4.49±0.90	4.50±1.98
***Capsicum annum* Linn. (Solanaceae)**	3.67±3.50	15.57±2.39	16.97±0.50	22.88±4.48	24.28±0.56
***Carica papaya* Linn. (Caricaceae)**	15.27±6.18	45.30±3.43	52.22±2.09	64.22±1.34	71.12±2.53
***Telfairia occidentalis* Hook (Cucurbitaceae)**	8.49±0.42	10.62±2.76	10.75±3.74	12.95±1.06	23.44±1.47

Table 6: Statistical Comparison Of DPPH Inhibition Of Extracts

Concentration(%)	0.2	0.4	0.6	0.8	1.0
carrot vs. pepper	3.67±3.50	15.57±2.39**	16.97±0.50**	22.88±4.48****	24.28±0.56****
carrot vs. pawpaw	15.27±6.18**	45.30±3.43****	52.22±2.09****	64.22±1.34****	71.12±2.53****
carrot vs.fluted gourd	8.49±0.42	10.62±2.76	10.75±3.74	12.95±1.06	23.44±1.47****
pepper vs. pawpaw	15.27±6.18*(No)	45.30±3.43****	52.22±2.09****	64.22±1.34****	71.12±2.53****
pepper vs. fluted gourd	8.49±0.42	10.62±2.76	10.75±3.74	12.95±1.06*(No)	23.44±1.47
pawpaw vs. fluted gourd	8.49±0.42	10.62±2.76****	10.75±3.74****	12.95±1.06****	23.44±1.47****

(Tukey's Two-way ANOVA analysis, 99% Confidence Interval, p<0.01)
Data expressed as Mean±SEM at 5 concentrations.
*(No)= Not significantly different from first extract at p<0.01 (p <0.05)
**= Significantly different from first extract at p<0.01(p<0.01)
**** = Significantly different from first extract at p<0.01(p<0.0001)

Table 7: IC_{50}values of Plant Extracts

PLANT EXTRACT	IC_{50} VALUES
***Daucus carota* Linn. (Apiaceae)**	**12.2325**
***Capsicum annum* Linn. (Solanaceae)**	**1.9734**
***Carica papaya* Linn. (Caricaceae)**	**0.6057**
***Telfairia occidentalis*Hook (Cucurbitaceae)**	**2.8805**

Table 8: Carotenoid Content Of Plants In This Study Compared With Literature

PLANTS	TCC in this study (µg/g)	TCC in recent works (µg/g)	References
DC	0.8425	295	Mech-Nowak *et al*., (2012)
CA	101.6167	187-10,121	Wall *et al*., (2001)
CP	8.7425	38.6 (β-carotene)	Maisarah *et al.*, (2013)
TO	5.4550	989(β-carotene)	Badifu *et al.*, (1995)

DC= Daucus carota, CA= Capsicum annum, CP= Carica papaya, TO= Telfairia occidentalis

EFFECT OF EFFLUX PUMP ACTIVITY INHIBITION ON ANTIBIOTIC RESISTANCE OF BACTERIAL ISOLATES FROM PATIENTS ATTENDING LAGOS UNIVERSITY TEACHING HOSPITAL - A PILOT STUDY

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ABSTRACT

The faster rate of emergence of resistant bacteria through many mechanisms compared to the development of new antimicrobial drug molecules has led to increasing morbidity and mortality of patients. Consequently, this pilot study attempts to explore the possibility of inhibition of efflux pump activity (EPA) on the susceptibility of some multidrug resistant bacteria (MDR) isolated from patients attending Lagos University Teaching Hospital. This will provide basis for initiating research into formulation of antibiotics with Efflux pump inhibitor (EPI) to combatMDR bacteria using the EPA mechanism.

Sixty nine (69) clinical bacterial isolates were screened and five MDR Gram-positives and 4 MDR Gram-negatives detected were tested for EPA against four different antibiotics - amoxycillin, cefuroxime, ciprofloxacin and tetracycline to which they demonstrate resistance.The Minimum Inhibitory Concentrations (MICs) of these antibiotics were determined against these MDR organisms both in the absence and presence of 200µg/ml of the EPI used - Trifluoro-methylbenzyl piperazine (mTFMBP).

All the Gram-negative and Gram positive organisms tested showed efflux pump activity to ciprofloxacin, one each to cefuroxime, while 5 and 3 to tetracycline respectively. None of the organismsshowed EPA to amoxycillin. Also, the TFMBP used was found to lower significantly MICs of at least 2 to 3 of the drugs tested in this study.

Arising from this pilot study, it could be deduced that the antibiotic resistance of many MDR organisms using EPA resistance mechanism could be reversed. This is possible using suitable EPIs successfully formulated in combination with the antibiotics.

Keywords:*Multidrug resistance, Efflux pump activity.*

INTRODUCTION

The continued emergence of new resistant strains of bacterial organisms at a rate much faster than the rate at which new drug molecules are being developed is resulting in increasing morbidity and mortality in both the developing and the developed countries of the world (Fischbach & Walsh, 2009; Ogunsola, 2012).

Although Aibinu *et al.*, 2003, Iroha *et al.*, 2008 amongst others have reported the prevalence of Extended Spectrum Beta-Lactamase (ESBL) producing bacteria and Methicillin Resistant *Staphylococcus aureus* (MRSA) in secondary and tertiary hospitals in some Nigerian hospitals , yet it is believed that there is paucity of information on the mechanism of multidrug resistance (MDR) and their prevalence in Nigeria and sub-saharan Africa in general(Shittu *et al.*, 2011).

This situation is further complicated by the permeability barrier and efflux mechanisms that also affect different classes of antibiotics such as quinolones, aminoglycosides, and tigecycline(Hawkey & Jones, 2009).With active efflux pump, even normal clinical doses of an antibiotic will present as sub-clinical dose *invivo* thus encouraging emergence of resistant strains(Hawkey & Jones, 2009).

This pilot study therefore attempts to study the EPA mechanism of MDR activity of some clinical isolates of bacteria to the tetracyclines, quinolones and β-lactams and the effect of EPI in combating this.

Bacterial Resistance due to Efflux Pump Activity(EPA) :

Multidrug resistance exhibited by bacterial organisms to chemically unrelated antibacterial agents have been found to be caused by over expression of MDR efflux pumps in many bacterial organisms (Bohnert& Kern,2005). Efflux pumps are transport proteins involved in the extrusion of toxic substrates (including virtually all classes of clinically relevant antibiotics) from within cells into the external environment. These proteins are found in both Gram-positive and Gram-negative bacteria as well as in eukaryotic organisms (Lomovskaya *et al.,* 2001).(Lomovskaya *et al.,* 2001).

Pumps may be specific for one substrate or may transport a range of structurally dissimilar compounds (including antibiotics of multiple classes); such pumps can be associated with multiple drug resistance (Lomovskaya *et al.,* 2001). Also a single pump is able to confer resistance to multiple compounds (Stavri *et al*., 2007).

Although genes encoding efflux pumps can be found on plasmids, the carriage of efflux pump genes on the chromosome gives the bacterium an intrinsic mechanism that allows survival in a hostile environment e.g. the presence of antibiotics (Webber & Piddock, 2003).

Detection of Efflux Pump Activity using EPI.

In a study by Bohnert& Kern(2005), a number of arylpiperazines were studied for their ability to reverse multidrug resistance in *E. coli* over expressing RND efflux pumps. The compounds were screened by evaluating the MICs of levofloxacin alone and in the presence of putative EPIs in *E. coli* test strains over expressing acrAB and acrEF. They also tested the compounds further to see that their minimal concentrations required to reduce the levofloxacin MICs by fourfold was at least fourfold lower than the intrinsic MIC of the test arylpiperazine compound. Among the compounds tested, 1-[3-(trifluoromethyl)benzyl]-piperazine (mTFMBP) was found to give a fourfold MIC reduction of levofloxacin at 200µg/ml.They also confirmed that the 200µg/ml used was at least fourfold lower than the intrinsic MIC of the mTFMBP.

METHODS

Collection of isolates:

Clinical isolates used for the study were collected from the laboratory cultures obtained from specimen of patients attending Lagos University Teaching Hospital.

The sources of the specimenswereurinary tract infection, upper respiratory tract infection , gastro intestinal tract, skin,blood, eye swabs and ear swabs.

Sixty nine isolates were collected, characterised and identified using standard methods(API 20E, 2005; API Staph, 2009). Thereafter, antibiotics susceptibility testing was done to determine the MDR resistant species among the isolates. Strains found to be resistant to four or more antibiotics were regarded as multi drug-resistant. These MDRs were selected for Efflux Pump Activity (EPA) test mainly by determining the MICs of the test drugsto which the organisms showed resistance (amoxicillin, cefuroxime, ciprofloxacin and tetracycline) in the presence and absence of an Efflux pump inhibitor - Tetra-fluoro-methyl-benzyl piperazine (mTFMBP).

A reduction in MIC in the presence of the Efflux Pump Inhibitor (EPI) indicates resistance due to efflux pump activity (Bohnert& Kern, 2005).

Four MDR Gram negative and5 MDR Gram positive bacterial isolates detected were used for this study while *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923) were used as control organisms.

Based on the above, the EPA test was done on the resistant strains of the clinical bacterial isolates. Before carrying out the EPA on any of the isolates, it must be resistant to two or more of the selected antibiotics. Based on the aforementioned, the testing was performed as follows;

Antibiotics	**No. of Gram negative bacteria tested**	**No. of Gram positive bacteria tested**	**Total**
Amoxicillin	4	5	9
Cefuroxime	2	1	3
Ciprofloxacin	4	5	9
Tetracycline	4	5	9
Total	14	16	30

Determination of MIC using the broth macro dilution method:

The broth macro-dilution method was used to determine initial range of the MICs of these drugs against the bacterial isolates.

Procedure:

Four millilitres (mls) of sterile Mueller Hinton broth (MHB) was placed in each of 13 sterile test tubes. Fourmls of the stock solution of Tetracycline standard (2mg/ml) was placed in the first tube of MHB to effect a double dilution of the tetracycline to a concentration of 1mg/ml (1000µg/ml). From this tube a serial double dilution from one tube of MHB to the next was carried out up to the 11th tube to arrive at 0.9765µg/ml of tetracycline.

An 18hr fresh MHB culture of each strain was diluted to give a 1: 10^3 dilution in normal saline (equivalent to 0.5 McFarland standards). 0.1ml of the diluted normal saline culture was introduced into each tube from the 2^{nd} tube to the 12^{th} tube. The 12^{th} tube having no antibiotic was the growth control while the 13^{th} tube with only 4ml of sterile MHB served as the sterility control. All the tubes from the 2nd to the 13th were incubated at 35^0C for 18hrs (CLSI, 2008).

The MIC was thereafter identified by checking optical density (OD) at 550nm against an un-inoculated MHB tube as the reference blank. The first tube to record the least OD reading is the tube having the drug concentration taken as the MIC.

A similar procedure was carried out for each of the antibiotics tested.

Determination of MIC using the broth micro dilution method:

Starting in channel 1 with 3mls of 2000µg/ml of the stock drug solution, a serial double-dilution of each antibiotic was carried out in a twelve-channel Basin using 1.5ml of the diluent(either sterile phosphate buffer pH 6.0 for amoxicillin and cefuroxime or sterile water for tetracycline and ciprofloxacin) in channels 2 to 12.

From channel 1, 1.5ml was pipetted into channel 2 and mixed by blowing. Same procedure was followed up to channel 12 to give the required starting concentrations in 1.5ml in each channel.

The MDRs were subjected to MIC tests in the ninety-six well micro-titre plates used to prepare the culture medium containing different concentrations of the antibiotics in which the bacterial strains were grown. One microtitre plate was used to test one organism with 2 different drugs at a time and at ten different concentration levels. The wells in the eleventh and twelfth columns were used as growth control for the isolates and sterility control for the medium respectively. Two microtitre plates each were used for one organism to account for the 4drugs i.e.18 micro-titre plates in all. In each of the microtitre plates, two drugs were tested each in duplicate with and without EPI e.g. rows A and B for amoxycillin without EPI , rows C and D for the same amoxycillin in the presence of the Efflux Pump- Inhibitor Trifluoro-methyl-benzyl piperazine (TFMBP), while rows E and F were used for cefuroxime without EPI , and rows G and H for cefuroxime in the presence of the Efflux Pump Inhibitor - TFMBP. The second micro-titre plate was also used for ciprofloxacin and tetracycline in the same manner.

Procedure:
With the aid of the 12-channel multipipettor fitted with sterile tips, plates were prepared with required volumes of drug from the stock in the twelve channel basin, sterile MHB, EPI, and the I:3 dilution in normal saline solution of the overnight MHB culture of the test organisms were added into the corresponding rows of wells as in Table 1 below.
The plates were then covered and incubated at 35^0C for 18hrs. Thereafter, optical density (OD) of each well in the plates were read at 650nm wavelength using the Spectramax plus (384) plate reader.
OD readings consistent with those of the sterility control wells were considered the MIC points for the drugs against the bacterial strains being tested.

RESULTS
The result of the biochemical test (API) showed that the nine organisms tested were identified as follows (Table 2):

All the 4 (100%)MDR Gram-negative bacteria tested gave evidence of efflux pump activity (Table 3). One *K.pneumoniae* strain showed efflux pump activity to 3 of the drugs tested. While 3 (60%) of the MDR Gram-positive cocci tested gave evidence of efflux pump activity against at least two of the the tested antibiotics (Table 4).

DISCUSSION
The results from this pilot study has demonstrated the importance of EPA as a mechanism employed by microorganisms in resistance to antibiotics used in clinical management of patients in this part of the world. It was observed that significant efflux pump activity to at least one of the antibiotics tested was obtained from all the MDR Gram negative and Gram positive organisms tested.All the 4 MDR Gram negatives showed significant efflux pump activity to both tetracycline and ciprofloxacin while only 3 of the MDR Gram positivesshowed efflux pump activity to these antibiotics.
The MIC for ciprofloxacin was reduced 16-fold in three of the MDR Gram negative strains inthe presence of TFMBP and same result was observed with tetracycline and cefuroxime, though with lesser number of susceptible organisms in the presence of TFMBP. This indicates that the EPI alone not in combination with the drug cannot result in inhibition of the organism since the MIC of the EPI (mTFMBP) gave intrinsic value of >400µg/ml. However, when used at even lower concentration of 200µg/ml with the antibiotics, it resulted in decreased MIC thus showing that EPI in combination with antibiotics can help to reduce antibiotic resistance, more so, multidrug as demonstrated in this study. This observation is in consonance with the results obtained by El-Deeb *et al*., (2003) stating that compounds exhibiting MICs of more than 200µg/ml are generally considered as weak antibacterial agents and could be considered as potential source of efflux pump inhibitor.
The TFMBP used in this work was found to lower significantly MICs of up to 2 or 3 drugs in some of the MDRs tested. This is consistent with the report of Stavri *et al*. (2007) that a single efflux pump inhibitor can confer susceptibility to multiple compounds.
The MICs given by these organisms in the absence of TFMBP are obviously much higher than clinically useful *in vivo* concentrations. Albeit with active efflux pump, even normal clinical doses of an antibiotic will present as sub-clinical dose *in vivo* which is the cause of the emergence of resistant strains. Thus the effect of EPI cannot be overemphasized as seen in this report.

The presence of significant efflux pump activity in the MDRs tested in this study further underscores the need for research in combating MDR via the tackling of efflux pump activity in

resistant organisms. This is in agreement with the findings of Fiamegos et al. (2011) that one plausible antimicrobial alternative to MDR could be the combination of conventional antimicrobial agents/antibiotics with small molecules which block multidrug efflux systems known as efflux pump inhibitors.

Therefore, significant lowering of MICs obtained in this study in the presence of the EPI reveals that designing newer drugs or modification of existing drug molecules with the inclusion of EPIs is going to lead to the development of more potent compounds for tackling chronic diseases resulting from multidrug resistant organisms. Furthermore, some antibiotics such as tetracycline that have been abused and probably neglected due to resistance could be introduced through the advent of EPIs as seen in this pilot study. This correlates with the assertion of Stavri*et al.* (2007) that the use of bacterial resistance modifiers such as EPIs could facilitate the re-introduction of therapeutically ineffective antibiotics such as tetracycline into clinical use and might even suppress the emergence of more MDR strains.

CONCLUSION

Arising from this study it cannot be overemphasised that the effect of efflux pumps needs to be considered in the design of future antibiotics and the role of inhibitors assessed in order to maximize the efficacy of current and future antibiotics. Also, the finding that an efflux pump inhibitor is capable of reducing MICs of more than one antibiotic by 4 to 16-fold suggests a line of research into formulation of combination drugs for combating the scourge of MDRs in our environment.

RECOMMENDATIONS

It is therefore recommended that research with a focus towards the designing and formulation of newer drugs or modification of existing drug molecules with the inclusion of EPIs should be harnessed in earnest as this is going to lead to the development of more potent compounds for tackling MDRorganism already ravaging our environment.

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TABLE 1: Example of contents of microwell in the presence of 200µg/ml of TFMBP

	Channel No	1	2	3	4	5	6	7	8	9	10	11	12
MATERIAL IN	Drug Stock conc. (µg/ml)	500	250	125	62.5	31.25	15.625	7.813	3.9065	1.95325	0.97663	**Sterility Control**	**Growth Control**
DRUG (µl)		50	50	50	50	50	50	50	50	50	50	-	-
MHB (µl)		111	111	111	111	111	111	111	111	111	111	111	111
TFMBP Stock. (1179µg/ml) in µl.		34	34	34	34	34	34	34	34	34	34	-	-
Make up water/buffer (µl)		-	-	-	-	-	-	-	-	-	-	84	89
Bact. Culture (µl)		5	5	5	5	5	5	5	5	5	5	5	
Total Vol.(µl)		200	200	200	200	200	200	200	200	200	200	200	200
Final Drug conc. in well(µg/ml)		**125**	**62.5**	**31.25**	**15.625**	**7.81**	**3.91**	**1.95**	**0.98**	**0.49**	**0.24**	-	-

Table 2: API identification of the MDR organisms tested

Isolates Log Number	Shape and Gram reaction	API20E/APIStaph identification
OG1	Gram negative rod	*Kleb. pneumonia*
N21	Gram negative rod	*E.coli*
N14	Gram negative rod	*Enterobacter aerogenes*
N3	Gram negative rod	*Kleb. pneumonia*
ML15	Gram positive cocci	*Staph aureus*
ES14	Gram positive cocci	*Staph lentus*
ES 1	Gram positive cocci	*Staph aureus*
ES16	Gram positive cocci	*Staph xylosus*
ES 44	Gram positive cocci	*Staph aureus*

Table 3: Result of the efflux pump activity of the Gram negative organisms tested:

Isolates/antibiotics	N 3		OG 1		N14		N21	
	MIC with 200µg/ml of TFMBP (µg/ml)	MIC (without TFMBP) (µg/ml)	MIC with 200µg/ml of TFMBP (µg/ml)	MIC (without TFMBP) (µg/ml)	MIC with 200µg/ml of TFMBP (µg/ml)	MIC (without TFMBP) (µg/ml)	MIC with 200µg/ml of TFMBP (µg/ml)	MIC (without TFMBP) (µg/ml)
Amoxicillin	>500	>500	125	125	>500	>500	>500	>500
Cefuroxime	31.25	62.5	-	-	>500	>500	-	-
Tetracycline	125	500	31.25	125	31.25	125	15.625	125
Ciprofloxacin	3.91	62.5	1.95	7.81	0.245	3.91	7.81	15.625
Comment on EPI Activity	16-fold reduction in MIC of ciprofloxacin, 4-fold in tetracycline and 2- fold in cefuroxime		4-fold reduction in MIC of ciprofloxacin and 4-fold in tetracycline		16-fold reduction in MIC of ciprofloxacin and 4-fold in tetracycline		2-fold reduction in MIC of ciprofloxacin and 8-fold in tetracycline	
MIC of TFMBP used	>400		>400		>400		>400	

Table 4: Result of the efflux pump activity of the MDR Gram positive bacteria tested:

ISOLATES	ML 15		Es 1		Es14		Es 16		Es44	
	MIC with 200µg/ml of TFMBP (µg/ml)	MIC (without TFMBP) (µg/ml)	MIC with 200µg/ml of TFMBP (µg/ml)	MIC (without TFMBP) (µg/ml)	MIC with 200µg/ml of TFMBP (µg/ml)	MIC (without TFMBP) (µg/ml)	MIC with 200µg/ml of TFMBP (µg/ml)	MIC (without TFMBP) (µg/ml)	MIC with 200µg/ml of TFMBP (µg/ml)	MIC (without TFMBP) (µg/ml)
Amoxicillin	0.98	0.98	1.95	1.95	>125	>125	125	125	0.49	0.49
Cefuroxime	-		-		-		-		3.91	15.625
Tetracycline	31.25	31.25	15.625	31.25	15.625	62.5	15.625	62.5	62.5	62.5
Ciprofloxacin	3.91	7.81	7.81	15.625	0.245	0.98	0.245	0.98	0.49	0.49
EPI Activity	2-fold reduction in MIC of ciprofloxacin		2-fold reduction in MIC of ciprofloxacin and MIC of tetracycline		4-fold reduction in MIC of ciprofloxacin and MIC of tetracycline		4-fold reduction in MIC of ciprofloxacin and MIC of tetracycline		4-fold reduction in MIC of cefuroxime	
EPI- MIC	>400		>400		>400		>400		>400	

PHYSICOCHEMICAL AND QUANTITATIVE ANALYSIS OF TWELVE BRANDS OF LEVOFLOXACIN TABLETS OBTAINED FROM A COSMOPOLITAN CITY

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ABSTRACT

Background: The spread of substandard drugs is a major health problem in both the developing and the developed countries. This is on the increase, partly as a result of the increase in the number of generic substitutes introduced into the drug market. **Aim:** This study was aimed at assessing the physicochemical and quantitative quality of randomly selected brands of Levofloxacin tablets marketed in Lagos, Nigeria. **Methods:** The methods used for the uniformity of weight, friability and disintegration test were obtained from the British Pharmacopoeia (BP) and the United States Pharmacopoeia (USP). The ultraviolet/visible spectroscopic method was used for the quantitative assay. **Results:** The results showed that all the samples, L1 - L12 passed the uniformity of weight test. All the brands (100%) passed the hardness/crushing strength test, friability test and disintegration test. Using the BP specification (95-105%), all the samples passed the UV/Visible spectrophotometric analysis. **Conclusion:** In order to elicit maximum and desired therapeutic effect, drugs are required to fall within specified limits as stated in the pharmacopoeia. Any brand outside this range is termed sub-standard and this may result into therapeutic failure if the quantity is below that specified in the pharmacopoeia or toxicity if above the range. Interestingly, in this study all the twelve brands analysed passed the tests.

Key words: *levofloxacin tablets, physicochemical tests, quantitative analysis, UV/Visible spectrophotometry*

INTRODUCTION

The spread of fake and substandard drugs is a major health problem in both the developed and the developing countries. The existence of counterfeit and substandard drugs particularly antibiotics and antiparasitic agents have been increasingly reported in the developing countries.[1,2] Drugs for the treatment of serious diseases such as malaria, tuberculosis, AIDS or other infections are more often the object of counterfeit.[3,4] Consistent with the recommendation of the World Health Organisation (WHO) launched the International Medical Products Anti-Counterfeiting Taskforce (IMPACT) in February, 2006 to stop the production and trading of fake medicines.[2]

The oral route is most frequently used for introducing drugs into the body, and in fact the vast majority of drug dosage forms are designed for oral ingestion, primarily for ease of administration. Whenever a drug is ingested orally, one would like to have it absorbed into the bloodstream rapidly and completely.[5]

Tablets are the most frequently administered oral solid dosage form. The increase in the number of generic drug products from multiple sources has placed people involved in the delivery of health care in a position of having to select one from among several seemingly equivalent products. For instance, in 1975 approximately 9% of all prescription drugs dispensed in the

United States were generic versions.[6] This figure rose to 20% in 1984 and 40% in 1991. Over 80% of the approximately 10,000 prescription drugs available in 1990 were obtained from more than one source and variable clinical responses to these dosage forms supplied by two or more drug manufacturers was documented.[7] These variable responses may be due to formulation ingredients employed, methods of handling, packaging and storage and even the rigors of in-process quality control. Thus, in 2003, it was suggested that there is a need for determining the pharmaceutical and therapeutic equivalence of different brands of drugs in order to ensure interchangeability.[8]

However, many developing countries do not have an effective means of monitoring the quality of generic drug products in the market. This results in widespread distribution of substandard and/or counterfeit drug products. It was in view of this fact that the World Health Organization issued guidelines for global standard and requirements for the registration, assessment, marketing, authorization and quality control of generic pharmaceutical products.[9] This was to give technical guidelines to national regulatory authorities such as NAFDAC (National Agency for Food and Drug Administration and Control), which is responsible for drug administration and control in Nigeria, on the quality of drug dosage forms generally available in the market. Generic drug products must satisfy the same standards of quality, efficacy and safety as those applicable to the innovator products. Preliminary physicochemical assessment of the products is very important and *in-vitro* dissolution testing can be a valuable predictor of the *in-vivo* bioavailability and bioequivalence of oral solid dosage forms.[10]

Generic substitution is defined as dispensing of product that is generically equivalent to the prescribed product with the same active ingredients in the same dosage form, and identical in strength, concentration, and route of administration.[11] The indication for the use of generic names for drug purchasing as well as prescribing is precisely to facilitate drug substitution whenever appropriate. It has been stated that the use of generic names for drug purchasing and prescribing carries considerations of clarity, quality, and price and it forms one of the core drug use indicator for the assessment of rational prescribing behaviour in practical settings.[11,12]

Nevertheless, opponents of generic substitution argue that the quality of generic drugs might be inferior to that of brand name products.[12] It is therefore important to ensure that generic substitutes are bio-equivalent. This is particularly important for developing countries like Nigeria where drug distribution and supply is known to be erratic. Factors that often necessitate the need for adequate bioequivalence studies include treatment failures, high cost of patented products, increase in resistance strains and sub standardization.[13]

Levofloxacin is a fluoroquinolone. The fluoroquinolones are widely used synthetic antibacterial agents to rival the beta-lactam and the macrolide antibacterial for impact in clinical usage in the antibacterial therapeutic field. They have a broad antibacterial spectrum of activity against gram-positive, gram-negative and mycobacterial pathogens as well as against anaerobes. Furthermore, they show good-to-moderate oral absorption and tissue penetration with favourable pharmacokinetics in humans, resulting in high clinical efficacy in the treatment of many kinds of infections. They also exhibit excellent safety profiles comparable to those of oral beta-lactam antibiotics.[14].

Chemically, levofloxacin is a chiral fluorinated carboxyquinolone. It is the pure (-)-(s)-enantiomer and L-isomer of the racemic drug substance, ofloxacin. The chemical name is (-)-(s)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7hydro-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid hemihydrate. The molecule exists as a zwitterion at the pH conditions in the small intestine. The empirical formula is $C_{18}H_{20}FN_3O_4 \cdot ½ H_2O$, and the molecular weight is 370.38.[15] The chemical structure is shown in figure 1 below;

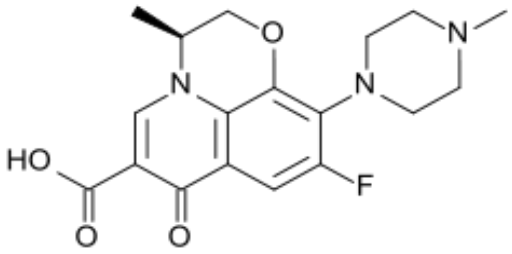

Figure 1: The chemical structure of levofloxacin.[16].

Levofloxacin is the L-isomer of the racemic drug ofloxacin. Like other fluoroquinolones, it inhibits both bacterial DNA gyrase and topoisomerase IV; the primary enzymatic target varies for different species of bacteria.[17,18] It has a broad spectrum of activity against gram-positive and gram-negative aerobes and atypical bacteria but it has limited activity against anaerobes.[19,20,21] It is active against both penicillin-susceptible and penicillin-resistant Streptococcus pneumonia.[16] It has an improved activity against streptococcus pneumoniae compared with ciprofloxacin or ofloxacin.[19]

In Nigeria, the drug regulatory authority and drug laws are in function, as a result the episode of substandard and fake drugs has reduced drastically.

In this study, the official methods in the pharmacopoeia and ultraviolet/visible spectrophotometry were adopted to ascertain the physicochemical and quantitative quality of the twelve brands of levofloxacin obtained from Lagos, Nigeria.

METHOD

Twelve brands of levofloxacin tablets were purchased randomly from Pharmacy outlets in Lagos, Nigeria.

Physical tests

The uniformity of weight test, disintegration test and friability tests were carried out according to the British Pharmacopoeia method (British Pharmacopoeia, 2008). The hardness test was performed using the method described by Rudnic and Schwartz.[22]

Quantitative assay-Ultraviolet/Visible spectrophotometry

Ten milligram (10mg) of the standard Levofloxacin pure sample (99.8% purity, courtesy Ranbaxy Nigeria Limited) was weighed and dissolved in a little quantity (5ml) of 0.1N HCl (prepared using B.P) and was then made up to 10ml. This sample was suitably diluted serially to different concentrations of 2, 4, 6, 8 and 10ug/ml respectively.[23] The absorbance of the above various dilutions were determined using UV/Visible spectrophotometer (T80+UV/VIS Spectrophotometer, PG® Instruments LTD, U.K) against a reagent blank (0.1N HCl) at λ_{max} 294nm. A graph of absorbance against concentration was plotted to get a calibration curve using Microsoft Excel.

Twenty (20) tablets from each brand were weighed and triturated into powder form in a mortar. A quantity of the powdered sample equivalent to 0.01g of Levofloxacin was weighed and dissolved in a little quantity (5ml) of 0.1N HCl and was made up to 10ml. Further dilutions were carried out and a final working solution of 8µg/ml was made. This procedure was repeated. The absorbance of each brand was then measured against a reagent blank (0.1N HCl) at λ_{max} 294nm. The concentration of each brand was derived from the regression equation obtained from the calibration plot using excel Microsoft office, 2010. The percentage purity of each levofloxacin brand was calculated and compared with the official specification.

RESULTS

Table 1: The physicochemical and quantitative result obtained for the twelve samples of levofloxacin tablet brands analysed.

Sample	% co-efficient of variation (Weight uniformity)	Friability (%)	Average Hardness (kg/cm)	Mean Disintegration Time (Min) ± SD	% Purity
L1	2.1000	0.1333	16.13+0.0579	19.26 ± 5.49	103.03
L2	1.5546	0.2400	12.97+2.2284	15.83 ±1.62	100.60
L3	0.6595	0.0598	15.13+0.0579	7.20±1.95	101.21
L4	1.8343	0.0754	13.73+1.1845	20.03±2.66	99.69
L5	3.3907	0.0322	14.00+1.3229	9.81±1.83	100.98
L6	1.6529	0.0819	11.83+0.2887	10.31±0.49	100.07
L7	0.7124	0.4738	16.03+0.0579	13.60±1.77	103.48
L8	1.6974	0.1590	14.13+0.7095	9.19 ±1.19	102.12
L9	1.5767	0.1832	15.70+0.2000	9.87±2.93	102.04
L10	2.4465	0.0518	15.00+0.0000	9.52±2.78	101.51
L11	0.9808	0.0800	15.60+0.1732	19.52±3.52	95.00
L12	2.5598	0.0593	11.53+1.6166	10.35±4.46	101.36

DISCUSSION

Quality control (QC) is part of quality assurance (QA) concerned with sampling, testing and documentation before, during and after manufacturing. Quality control is the monitoring process through which the manufacturer measures actual quality performance, compares it with standards and finds out the causes of deviation from the standard to ensure quality product, not once, but every time. In general terms, it refers to a procedure or a set of steps taken during the manufacturing of a product to ensure that it meets requirements and that the product is reproducible.

This research work focused on the evaluation of twelve brands of levofloxacin hemihydrate tablets marketed locally in Lagos, Nigeria. All the brands used for this evaluation were obtained from different community Pharmacy premises.

A number of research work done in other countries that indicate the physicochemical parameter evaluation of different drugs are available but very scanty information is available in our country.[24,25,26,27,28,29,30] These parameters are required for the achievement of a stable and effective drug product.

The uniformity of weight test is one of these physical tests described in the compendia and this is used for ascertaining that the quantity of the granulation which contains the labelled amount of therapeutic ingredient is correct. The average weight of the levofloxacin tablet is more than 250mg, as such, in accordance with the compendia, not more than two (2) tablets must have a percentage deviation above ±5% and none of the tablet must have a percentage deviation above ±10%. From this evaluation, the results obtained for all the brands were within the specified limit (Table 1).

The hardness test or crushing strength for tablets is a non-compendia test and there is no official standard for mechanical strength of tablets. However, manufacturers normally employ test to ensure that their tablets will withstand the normal risks of breakage under conditions of storage, handling and transporting. These are either simple qualitative test for wear and tear or test for

strength using mechanical devices of some kind. Before the use of drugs, this parameter should be evaluated.
For this evaluation, the Ketan hardness tester was used and it measures the degree of hardness of the tablet in kg/cm. A hardness of 4kg/cm is considered to be the minimum for a satisfactory hard tablet. If the tablet is too hard, it may not disintegrate within the required period of time or meet the dissolution specifications. However, the same hard tablet may be formulated with a very good disintegrating agent (an excipient) and this enhances the disintegration and dissolution of the tablet when in contact with water. If the tablet is too soft, it will not withstand the handling during subsequent processing and shipping. Oral tablets normally have a hardness of 4-10kg/cm, hypodermic and chewable tablets are much softer (3kg/cm) and some sustained release tablets are much harder (10-20kg/cm).[22]

From Table 1, the range of hardness for the levofloxacin tablet analysed is 11.53kg/cm-16.13kg/cm. Levofloxacin tablet being a once daily dosed tablet, is formulated as a relatively slow release tablet so as to sustain its level in the system for 24 hours. All the brands of levofloxacin analysed have a crushing strength between 10-20kg/cm and in this study, they all passed the hardness test.
Friction and shock are the most often conditions that cause tablets to chip, cap or break. Rocha friabilator was used for this test. The tablets roll and fall within the apparatus and the percentage tablet loss is measured (a measure of the degree of inter-particulate attraction). It is believed that the stronger the strength of the bond; the lower the friability of the drug. The United State Pharmacopoeia (USP) 27th edition stated that the percentage friability of tablets should not be more than 1%. The result of tablet friability test in Table 1 showed that all the tablet samples tested exhibited an impressive friability value ranging from 0.0322%w/w to 0.4738%w/w which is less than 1%.

Disintegration test was first introduced in the 7th (1945) addendum of the BP 1932 and was modified several times until a test closely resembling the current procedure was introduced in 1955. This test measures the time required for tablets to disintegrate into particles. This is a necessary condition for dissolution and could be the rate determining step in the process of drug absorption. From Table 1, all the samples passed the British Pharmacopeia BP specifications for disintegration rate test. The disintegration rates ranged from 7.20±1.95 to 20.03±2.66 minutes, indicating that all the disintegration times were within the BP official specification of 30 minutes.[23] The various brands could have employed disintegrating agents to improve the penetration of aqueous liquids. The addition of disintegrating agents (e.g., starch, methylcellulose) in the right proportion yields tablet products free of disintegration problems.[31]
The chemical assay of the drug describes the test in which the content (active moiety) of the drug sample is checked to make sure it conforms with the standard. Every unit of tablet should contain the same amount of drug substance equivalent to its label content. A key component of the overall quality of pharmaceutical product is control of impurities, presence of therapeutic agent that developed potency, safety and efficacy of drug. Different kind of analysis can be employed for analysis, however, in this study; the evaluation of content of the levofloxacin tablets was carried out using UV/Visible spectrophotometry.

Adulteration is defined as the alteration of any substance by the delibrate addition of a component not ordinarily part of that substance, usually used to imply that the substance is debased as a result. A fake drug or counterfeit medication or pharmaceutical product is one which is produced and sold with the intent to deceptively represent its origin, authenticity or effectiveness. This may contain inappropriate quantities of active ingredients, or none, or may contain ingredients that are not on the label which may or may not be harmful. As opposed to a

fake drug that sometimes contains harmful ingredients; a substandard drug simply does not produce the desired medical benefit as a result of the drug not containing the exact amount of ingredient as supposed by the official book. However, if this is above what is specified, it may lead to toxicity.

According to BP specification, the assay of the active chemical content of the levofloxacin tablets must fall within 95% and 105%. Although different manufacturers formulate the different brands of levofloxacin tablet by different methods, all should fall under the BP/USP specifications. From this analysis, all the brands of levofloxacin analysed passed the quantitative assay (Table 1). The % purity obtained for the twelve brands ranged from 95% to 103.48%.

CONCLUSION

Pharmaceutical quality control and quality assurance depend on monitoring the composition and uniformity of the drug substance during processing and in the final product. Compendia tests have been used traditionally to determine identity, strength, quality, and purity of drug dosage forms. Implementation of these approaches can reduce the time and cost required for manufacturing, while improving quality control.

The hardness test which is a non-compendia test showed that all the brands passed the test. The twelve brands also passed the uniformity of weight, friability and the disintegration test which are compendia tests. All the samples of levofloxacin analysed passed the quantitative test ranging from 95.0%-103.48%.

The twelve brands of levofloxacin analysed were in conformity with the set standards, this might be as a result of the intense campaign and hard work that has been carried out by National Agency for Food, Drug Administration and Control (NAFDAC) in the country to sanitize the drug market.

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ASSESSING BELIEFS ABOUT MEDICATIONS FOR HYPERTENSION AND THEIR IMPACT ON ADHERENCE

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ABSTRACT
Background
Poor adherence to medication among hypertensive patients has been documented and shown to be associated with poor outcomes, progression of disease state/ associated target organ damage, reduction of quality of life and death. In assessing adherence, there is no gold standard; structured questionnaires are convenient tools in assessing a patient's adherence and can identify influential beliefs that predict medication taking behaviors. **Method:** An exploratory survey among hypertensive patients was carried out at one pharmacy, two secondary hospitals and a tertiary hospital in Lagos state. Two research instruments were utilized: pre-validated Morisky Medication Adherence Scale (to measure self-reported adherence) and the Hypertension Health Belief Questionnaire (to measure hypertension medication taking beliefs). The Medicines for Hypertension Questionnaire was developed from Farmer, Kinmonth and Sutton's Diabetes Belief Questionnaire with the aid of interviews guided by the theory of Planned Behavior. Ethical approval was sought and received from LUTH Health Research and Ethics Board and the Lagos State Health Management Board. **Results:** Questionnaires were returned by 493 (91.3%) people. Positive behavioral beliefs about medication taking behavior were widely held but not significantly associated with self-reported medication adherence. Normative beliefs and control beliefs about medication taking behavior were all significantly associated and correlated with self-reported adherence ($p< 0.001$). Intention to use medication was not associated with beliefs about benefits. Negative behavioral beliefs were significantly associated with adherence, and belief that "change to daily routine will make it more difficult to adhere" were associated with reduced adherence. **Conclusion:** Patient's belief about medication taking behavior significantly modifies their medication adherence and informs an intervention model that can be used regularly to identify and resolve concerns. These beliefs and their associated effect on adherence are clinically relevant and form a basis for further exploration.

Keywords: *Hypertension, Planned behaviour, adherence, belief and medication.*

INTRODUCTION

Hypertension is the most diagnosed non-communicable disease in adults (>18years) and its incidence is on the increase worldwide (WHO, 2012)., it is defined as ≥ 140mmHg systolic and/or ≥ 90mmHg diastolic blood pressure measured in at least two clinic visits. Hypertension in about 90-95% of patients is multi-causal and the primary cause cannot be identified (primary hypertension). It usually results from interplay between genetic factors and a myriad of environmental factors. Secondary hypertension most commonly results from renal dysfunction due to primary chronic kidney disease or reno-vascular disease (Chobanian et al, 2003). The risk of a hypertensive patient developing stroke, myocardial infarction (MI), angina, heart failure or sudden death is directly correlated with blood pressure levels (Lewinton et al, 2002). The overall goal of management of hypertension is the reduction of hypertension associated morbidity and mortality (Chobanian et al, 2003).

The WHO defined adherence as "the extent to which a person's behaviour – taking medication, following a diet, and/or executing lifestyle changes, corresponds with agreed recommendations from a health care provider" (Sabaté, ed. 2003; Brown and Bussell, 2011). Adhering to medical and non-medical recommendations from healthcare providers has been associated with improved health outcomes in patients with hypertension alone or with a co-morbid disease. There is no consensus on interventions to positively influence adherence to medical and non-medical recommendations due to varying reports of effectiveness and non-standardized methods used in many interventions (Ebrahim, 1998; Sabaté ed. 2003).

Assessment of adherence behaviour is necessary for planning effective and efficient treatment and also for ensuring that changes in health outcomes are attributed to the recommended regimen for example, it has been shown that high adherence to antihypertensive regimen is directly associated with reduction in morbidity and mortality (Morisky et al, 2008; Kroussel-Wood et al, 2009). There is currently no gold standard in the measurement of adherence behaviors but self-reported measures have been identified to be appropriate for routine clinic use (Sabaté, ed. 2003; Fairman and Motheral, 2000). The literature reports that less than half of the patients seen for follow-up adhere to their medical recommendations up to 80% of the time (Sackett, et al, 1975; Atreja, Bellam and Levy, 2005). Studying medication-taking behaviours as influenced by beliefs may yet offer the key to unlocking the potential reduction in morbidity and mortality that seems elusive. The theory of planned behaviour itself explores beliefs about a targeted behaviour (eg medication taking behaviour) and has been shown to have predictive validity, explaining variance in intention and behaviour across a wide range of different target behaviours (Farmer, Kinmonth and Sutton, 2005). Exploring these medication taking beliefs may be important in designing interventions to improve adherence. and pharmacists are well positioned to utilize these instruments during their contact with patients.

METHOD

Study Design and Setting

The study was done in Lagos, South-West Nigeria. Lagos is a port city and the most populous city in Nigeria. The city of Lagos is divided into the "Mainland and Island" with 20 Local Government Areas and 37 Local Council Development Areas (Lagos State Government, 2011). This study was carried out at one pharmacy, two secondary hospitals and one tertiary hospital for convenience.

Sample Selection and Sampling

Patients recruited and surveyed had been diagnosed hypertensive in a hospital and had been prescribed at least one antihypertensive medication for at least six months duration. Patients with co-morbid diseases like diabetes, stroke, myocardial infarction, arthritis were excluded from the study due to higher pill burdens in these populations.

Eligible patients were identified by general practitioners/ nurses during presentation at the clinic or by pharmacist during a refill and referred to the investigator. Consent was obtained by completing a consent form attached to the questionnaire. While every consenting patient was given a copy of the Morisky Medication Adherence Scale 8 form to complete, only every third patient referred was administered the Medicines for Hypertension Questionnaire to add a measure of randomization to the selection process. Patients who could not read were assisted by trained research assistants who read out the forms in relevant language.

Ethical approval was sought and received from LUTH Health Research and Ethics Board and the Lagos State Health Service Commission.

Instruments

Two instruments were utilized in the survey, including The Morisky Medication Adherence 8 form and the Medicines for Hypertension Questionnaire.

Morisky Medication Adherence Scale 8 (MMAS-8) self-report adherence measure was designed to facilitate the identification of adequate adherence to chronic medications. In a previous study, the scale has been determined to be reliable (Cronbach alpha= 0.83) and significantly associated with blood-pressure control ($P< 0.05$) in low income, mostly minority, and under-served individuals with hypertension (ie, low adherence levels were associated with lower rates of blood-pressure control (Morisky et al, 2008)). The questions comprising the scale include: 'Do you sometimes forget to take your high blood pressure pills?' 'People sometimes miss taking their medications for reasons other than forgetting. Thinking over the past two weeks, were there any days when you did not take your high blood pressure medicine?' 'Have you ever cut back or stopped taking your medication without telling your doctor, because you felt worse when you took it?' 'When you travel or leave home, do you sometimes forget to bring along your high blood pressure medication?' 'Did you take your high blood pressure medicine yesterday?' 'When you feel like your high blood pressure is under control, do you sometimes stop taking your medicine?' 'Taking medication everyday is a real inconvenience for some people. Do you ever feel hassled about sticking to your blood pressure treatment plan?' 'How often do you have difficulty remembering to take all your medications?'

The Medication for Hypertension Questionnaire was developed from the Medicines from Diabetes Questionnaire (MDQ) by Farmer, Kinmonth and Sutton (2005). Interviews guided by the MDQ were conducted with 10 patients at the outpatient department of LUTH to identify beliefs about hypertension medicine taking behaviors and the recording was used to modify the MHQ. The final questionnaire was then modified for simplicity from the likert scale to a three point scale from 'Yes', 'I don't Know' and 'No'. Unlike in Farmer, Kinmonth and Sutton (2005), only 'I intend to take my hypertension medicines regularly' was included as a measure of intention. The exploratory questions include Behavioral beliefs preceded by the line If I were to take my high blood pressure medicines regularly …'It would help me to stay well and probably live longer', 'It would reduce my chances of developing complications from hypertension', 'It would keep my blood pressure under control', 'It would help me avoid having to be hospitalized for emergency services', 'It would cause me unpleasant side effects', 'This would lead to lower sexual satisfaction', Normative beliefs 'My doctor would approve of me taking my high blood pressure medicines regularly', 'Members of my family or close relatives would approve of me taking my high blood pressure medicines regularly', 'My wife/husband/partner would approve of me taking my high blood pressure medicines regularly', and Control beliefs 'Changes to my daily routine would make it more difficult for me to take my high blood pressure medicines regularly', 'Putting out my tablets in a box would make it easier for me to take my high blood pressure medicines regularly', 'Having a regular review with the doctor would make it easier for me to take my high blood pressure medicines regularly', 'Keeping to a regular routine and being disciplined would make it easier for me to take my high blood pressure medicines regularly'.

Data Collection and Analysis

All forms were filled and returned at the clinic or at the pharmacy. Responses from the questionnaire were entered into the SPSS 17 and verified. No identifying data was retained from the questionnaire as only serial numbers were assigned to the questionnaire themselves. SPSS 17 was utilized in analyzing the data obtained from the survey.

RESULTS

Demography

Females constituted the majority with 72.2%, 60% of the sample were aged 41-65 years and 86% claimed to have no health insurance/ benefit of any kind. 12.2%, 28.2%, 28.8% and 26.4% had post-secondary, secondary, primary and no education at all (respectively). The mean age of respondents who took part in this study was 57.53 years (Table 1).

Self-Reported Adherence

Average self-reported adherence in the sample was 6.3121 (±1.5672), a medium score of self-reported adherence (6-<8). Females were 1.69 (OR) times more likely to have high adherence as defined by the MMAS 8 scale than men in this sample. Patients who reported not having any health benefits were 1.34 (OR) times more likely to report high adherence than those with health benefits.

Medicines for Hypertension Questionnaire

Majority of respondents, over 80% agreed with the statements about positive behavioural beliefs ('It would help me to stay well and probably live longer', 'It would reduce my chances of developing complications from hypertension', 'It would keep my blood pressure under control', 'It would help me avoid having to be hospitalized for emergency services') about taking medicines for hypertension. The statements about negative behavioural beliefs were however not commonly held; 'It would cause me unpleasant side effects' was reported in 26% of the respondents while 'This would lead to lower sexual satisfaction' was held in 14.8% of respondents.

The Normative beliefs were also widely agreed to, with over 72% agreeing with the positive normative behavioural beliefs about taking medications for hypertension 'My doctor would approve of me taking my high blood pressure medicines regularly', 'Members of my family or close relatives would approve of me taking my high blood pressure medicines regularly', and 'My wife/husband/partner would approve of me taking my high blood pressure medicines regularly'.

A minority of respondents (32%) agreed to the statement 'Changes to my daily routine would make it more difficult for me to take my high blood pressure medicines regularly', while 47% of respondents agreed that 'Putting out my tablets in a box would make it easier for me to take my high blood pressure medicines regularly', more than 80% of respondents agreed with the two statements; 'Having a regular review with the doctor would make it easier for me to take my high blood pressure medicines regularly' and 'Keeping to a regular routine and being disciplined would make it easier for me to take my high blood pressure medicines regularly'.

Respondents who indicated positive responses to the statement of intention, 'I intend to take my hypertension medicines regularly' were 1.121 (OR) times more likely to have reported high adherence than medium and low self-reported adherence in this study. Association between the intention to take medication for hypertension and the self-reported adherence was significant (χ^2=7.438, $p< 0.05$).

Correlation between Behavioural Beliefs and Intention

All the positive and negative behavioural beliefs and normative beliefs explored did not show a significant association with intention to use medicines for hypertension in this study. However, two control beliefs 'Keeping to a regular routine and being disciplined would make it easier for me to take my high blood pressure medicines regularly' and 'Putting out my tablets in a box would make it easier for me to take my high blood pressure medicines regularly' were significantly associated with intention to take medicines for hypertension ($p< 0.001$ and $p< 0.05$ respectively).

Correlation between Behavioural Beliefs and Adherence

The two negative behavioural beliefs ('It would cause me unpleasant side effects' and 'This would lead to lower sexual satisfaction') showed statistically significant inverse associations with self-reported adherence ($p < 0.0001$). The positive behavioural belief statement 'It would help me avoid having to be hospitalized for emergency services' was also significantly associated with adherence ($\chi^2=30.973$, $p= 0.01$). All the normative beliefs and control beliefs also showed statistically significant associations with self-reported adherence ($p < 0.0001$).

DISCUSSION

In adapting the MDQ developed by Farmer, Kinmonth and Sutton (2005), considerations were given to developing an exploratory instrument that is valid and could be usable in the Nigerian healthcare system- simple enough to allow translations to the local language during use without ambiguity or loss of consistency. The use of structured interviews allowed for concise and relevant issues to be explored without losing focus during the interview sessions. This method while not exhaustive, aided the identification of the most common beliefs about medicines for hypertension among diagnosed patients who are currently receiving at least one anti-hypertensive medication at the surveyed sites.

Self-reported adherence in the sample studied was less than optimal; high adherence scores (8 on the MMAS8) has been reported to be correlated significantly with blood pressure control, reduced cardiovascular morbidity and mortality (Morisky et al, 2008; Kroussel-Wood et al, 2009). Medium scores (6 to <8 on the MMAS8) on the MMAS8 has not yielded consistent results in ameliorating risks of cardiovascular events. 61% of respondents had at least a medium score, this is comparable to that obtained by Lee et al (2013) in a Chinese population and lower than in Hyre et al (2007) in a US population. This level of adherence is as postulated in Caro and Payne (2000) and Sabaté (ed, 2003). There is currently no gold-standard in measuring adherence (Sabaté ed, 2003) hence, comparing adherence data may be cumbersome and erroneous except the same instrument was used and was interpreted in the same way.

Several studies have tried to elucidate the sample characteristics responsible for and associated with antihypertensive drug adherence. In this study, a chi-squared test showed significant relationship exists for age and self-reported adherence; and income and self-reported adherence. This is similar to findings in Lee et al (2013), Sabaté (ed. 2003), Ekwunife, Udeogaranya and Adibe (2010) and Okoro and Ngong (2012). Though these relationships are further moderated by other factors (Sabaté ed, 2003), they represent an important cue for healthcare providers during contact with patients.

Unlike in Farmer, Kinmonth and Sutton (2005), intention to use medicines for hypertension was not associated with positive beliefs about clinical benefits of taking medication but was significantly associated with two control beliefs. It is assumed that due to the asymptomatic nature of hypertension, patients may be demotivated to seek or continue on treatments that have side effects (Sabaté ed, 2003). Two negative behavioural beliefs and two control beliefs are associated with lower reported medication adherence. This exploratory study helps to identify beliefs for further exploration and cues for pharmacist/ other healthcare providers' intervention in clinical settings to improve adherence to medications prescribed.

In holding with the theory of planned behaviour, two control beliefs 'Putting out my tablets in a box would make it easier for me to take my high blood pressure medicines regularly' and 'Changes to my daily routine would make it more difficult for me to take my high blood pressure medicines regularly' showed association with both intention ($p < 0.05$) and self-reported adherence ($p < 0.0001$). Similar to findings in Farmer, Kinmonth and Sutton (2005), some behaviors were associated with intention but not with self-reported adherence while some were

associated with self-reported adherence but not with intention. It is possible to imagine that medication taking behaviours are modified by negative behavioural beliefs at the point of execution.

CONCLUSION

The adherence in this sample was not adequate to achieve the goal of consistently reducing cardiovascular morbidity and mortality. Targeted interventions using psychometric scales like this should be carried out during every patient's contact with the pharmacist or other healthcare providers; this will result in better targeted interventions to improve adherence to both pharmacological and non-pharmacological recommendations.

The Medicines for Hypertension Questionnaire was developed using the theory of planned behaviour and has been shown to be able to explore beliefs about medication taking behaviour in patients diagnosed with hypertension. The range of beliefs explored here are those commonly held by patients who are currently prescribed medicines for hypertension.

Limitations

While the Medicines for Hypertension Questionnaire was easy to use, it is assumed that some qualities for representativeness may have been lost to the three item 'Yes', 'No' and 'I don't know' responses used instead of likert scales in eliciting response to medication taking belief variables. Also making the measure for intention a single question item may have reduced the sensitivity of the instrument at finding some meaningful associations between intention, self-reported adherence and medication taking behaviour.

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Table 1: Beliefs About Taking Medicines for Hypertension, Percentage of Positive Responses, and Chi-Squared Test of Association with Intention and Self-Reported Adherence

Variables	Percentage of Positive Response %	Intention χ^2	Adherence χ^2
Behavioral beliefs			
It would help me to stay well and probably live longer	95.7	2.224 (p> 0.05)	3.97 (p> 0.05)
It would reduce my chances of developing complications from hypertension	85.2	1.441 (p> 0.05)	3.517 (p> 0.05)
It would keep my blood pressure under control	89.8	2.016 (p> 0.05)	2.711 (p> 0.05)
It would help me avoid having to be hospitalized for emergency services	83.4	1.093 (p> 0.05)	13.385 (p= 0.01)
It would cause me unpleasant side effects	26.5	1.793 (p> 0.05)	30.973 (p< 0.0001)
This would lead to lower sexual satisfaction	14.8	1.126 (p> 0.05)	30.657 (p< 0.0001)
Normative beliefs			
My doctor would approve of me taking my high blood pressure medicines regularly	82.6	1.610 (p> 0.05)	38.868 (p< 0.0001)
Members of my family or close relatives would approve of me taking my high blood pressure medicines regularly	74.5	4.295 (p> 0.05)	25.532 (p< 0.0001)
My wife/husband/partner would approve of me taking my high blood pressure medicines regularly	77.3	1.355 (p> 0.05)	26.908 (p< 0.0001)
Control beliefs			
Changes to my daily routine would make it more difficult for me to take my high blood pressure medicines regularly	32.0	7.357 (p< 0.05)	49.86 (p< 0.0001)
Putting out my tablets in a box would make it easier for me to take my high blood pressure medicines regularly	47.3	6.137 (p< 0.05)	43.58 (p< 0.0001)
Having a regular review with the doctor would make it easier for me to take my high blood pressure medicines regularly	85.8	0.051 (p> 0.05)	23.611 (p< 0.0001)
Keeping to a regular routine and being disciplined would make it easier for me to take my high blood pressure medicines regularly	80.8	3.857 (p> 0.05)	29.092 (p< 0.0001)

HEALTH PROMOTION PRACTICES AMONG COMMUNITY PHARMACISTS IN LAGOS STATE, NIGERIA: PERCEIVED IMPORTANCE AND INVOLVEMENT

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ABSTRACT

Aim: To investigate how much of importance is attached to the promotion of certain health-related behaviours by Community Pharmacists in Lagos State and establish the correlation between the level of importance attached to each health-related behaviour and their perceived extent of involvement in promoting it. **Method**: A cross-sectional survey of 150 randomly selected registered community pharmacists practicing within Lagos State was carried out. The level of importance pharmacists attached to health related behaviours was assessed using a 22-item instrument on a 5-point scale. (Very unimportant, unimportant, uncertain, important and very important).The same instrument was used in assessing their perceived degree of involvement in the promotion of these health behaviours. The average score for each health related behaviour was obtained for both variables (importance attached and involvement) and Pearson's correlation coefficient was used to establish the association between the two variables. **Results**: Apart from the three medication-related behaviours on the list, the only other item that received a "very important" rating by over 60% of the respondents was "the use of condoms if one has multiple sex partners". The average mean score for the level of importance attached and degree of involvement were 4.35 and 4.00 respectively on a scale of 5.0. A Pearson product-moment correlation showed there was a strong, positive correlation which was statistically significant ($r = .869$, $n = 22$, $p < .0005$). Impatient clients and time constraints were the two major barriers highlighted by over 60% of the respondents and almost all the respondents (99%) would be willing to participate in health promotion training. **Conclusion**: The level of importance attached to promoting health-related behaviours among community Pharmacists in Lagos is high and there is a strong positive relationship between the level of importance attached to a particular behaviour and the degree of involvement in promoting it. Community Pharmacists in Lagos State should be encouraged to be involved in health promotion practices to boost health services in the State.

Keywords*: Health promotion practices, Community Pharmacists, Perceived Importance, Involvement, Lagos State.*

INTRODUCTION

Health Promotion comes with many definitions but the most widely accepted one is enshrined in the Ottawa Charter which was a product of the first International Conference on Health Promotion. It defines Health promotion as "the process of enabling people to increase control over, and to improve, their health" [1]. Research and case studies from around the world provide convincing evidence that health promotion is effective in disease prevention.[2]

In history, Health Promotion has been traced to the earliest civilization. Johnson and Breckon noted that the fundamental needs of shelter, food, water, and safety are health related; even the writings of the Babylonians, Egyptians, and Old Testament Israelites indicate that various health promotion techniques were utilized.[3]

Health promotion practices are activities or efforts that are geared towards enabling or empowering people to increase control over their health and its determinants, and thereby improve their health.

Different kinds of activities have been carried out under the banner of health promotion, but It has been suggested that the primary criterion for determining whether a particular initiative should be considered health promoting, ought to be the extent to which it involves the process of enabling or empowering individuals or communities. The absence of empowering activities should therefore signal that an intervention does not fall within the rubric of health promotion.[4]
In sub-Saharan Africa, there is a rising profile of non -communicable diseases which has been reasoned to be as a result of changing lifestyles in the African society.

According to the World Health Organization, these lifestyle changes include; widespread tobacco, substance and alcohol use and abuse, reduced physical activity and consumption of diets rich in salt, sugar and fat, among others. These risk factors, along with mental and neurological disorders, are conspiring with injuries and violence to overstretch the fragile and already overburdened health systems in the African Region.[5] Health promotion would help stem this tide, it has been shown that "simple, cost effective public health measures could lengthen the average human life span by 5 to 10 years.[6]
Pharmacists especially community Pharmacists have great opportunity to make significant contributions to public health because the pharmacist has sufficient health knowledge on which to build and is often uniquely positioned in the community to provide public health services. The fact that no appointment is needed in most community pharmacies and consultations are usually free of charge further places the community pharmacist at an advantage in carrying out health promotion activities.

The practice of Pharmacy has changed significantly in recent years from the traditional focus on drugs alone to the interaction between the patient and the medication. This has been attributed to an increase in health demands, with a complex range of medicines for chronic diseases and poor adherence to prescribed medicines, which has forced pharmacists to take a patient-centered approach.[7] Today, the pharmacists' role in many practice settings has expanded to include not only dispensing functions, but also direct contact with patients and other providers.
In developing countries however, Azhar et al observed that pharmacists are still underutilized and their role as health care professionals is not deemed important by either the community or other health care providers.[8]

Levin, Hurd and Hanson observed that consumers are typically unaware of pharmacists' knowledge in other topics other than prescription drugs and that sometimes, consumers feel the pharmacist is too busy to have time for questions unrelated with the product being purchased. They further explained that from the pharmacists' perspective, involvement of more time, lack of reimbursement for these services, lack of training and formal recognition as health promoters may discourage pharmacists from health promotion activities.[9]
In the year 2000, Kotecki, Elanjian & Torabi assessed the Health Promotion beliefs and practices among Pharmacists in Indiana, USA, they concluded that while most pharmacists perceived that many health promoting behaviours are "very important" for the average adult, most did not feel they should be "very involved" in counselling patients on health-promoting behaviours.[10] They observed that that lack of involvement in health promotion activities by Pharmacists may be the result of not believing in the value of some of the behaviours they could promote or feeling confident in assisting their patients in behavioural change. They noted that personal beliefs concerning both the validity of health promotion and the pharmacist's ability to influence patient behaviour may affect how much effort a pharmacist spends on health promotion.[10]
Oparah & Okojie also worked on Health promotion perceptions among community pharmacists in Benin City, Nigeria**,** they observed that community pharmacists perceived an extended role in

health promotion, especially medication-related activities. Medication-related counselling, use of condoms, and maintenance of blood pressure were perceived to be top priorities. [11]

Another assessment of the community pharmacists in the state of Penang, Malaysia observed that a majority of the respondents (Pharmacists) were aware that health promotion is part of the Pharmacist's responsibility and that most of the community Pharmacists have provided health education and promotion programs for the public.[12]

Although several evidence-based studies have shown positive impact of community pharmacists in specific health promotion topics, Paluck, Stratton & Eni, reported that there is considerable room for increasing pharmacist involvement in health education and disease prevention activities.[13]

It has been suggested that Pharmacists have less interest in prevention activities because they feel these areas are too far removed from the traditional role of dispensing medication.[14]

In a systematic review of pharmacists' view of their public health role done by Eades, Ferguson & Caroll, they found out that most pharmacists viewed public health services as important and part of their role but secondary to medicine related roles.[15] They identified a number of common barriers to public health practice including availability of a private counseling area, time, customer demand/reaction and reimbursement for public health services.[15]

Hassali *et al* pointed out that ''lack of time'' and ''lack of specific training'' are ranked as major barriers to Pharmacists' involvement in health promotion practices. [12]

METHODS

Design of the study

The study was a cross-sectional survey on perceived importance and involvement of Community Pharmacists in Lagos State in Health Promotion practices.

Area & Population of the study

Licensed community Pharmacists practicing in Lagos State, Nigeria constituted the population of study. Lagos State is regarded as Nigeria's financial, commercial and industrial nerve centre. It has a population of over nine million people according to the National Population Commission.

Sample and Sampling Technique

The data obtained from the Pharmacist Council of Nigeria showed that 586 (five hundred and eighty six) registered pharmacy premises were in Lagos at the time of this study. The sample number was calculated on the assumption that there would be at least one pharmacist per premise.

The sample size was calculated to be 186 (Population size = 586, Margin error =5% and Confidence level =90 %.)

A Simple random sampling technique was employed.

Research Instruments

The research instrument used was a structured questionnaire. The questionnaire was divided into four sections. The first section captured the demographic details of the respondents.

The second section was to obtain the perception of community pharmacist about health promotion practices, 22 items that were suggestive of their attitude were used in measuring their perception Respondents were asked to state the extent to which they perceive that the health promoting behaviours were important or unimportant using a 5-point scale (very unimportant, unimportant, uncertain, Important and very important).This was adapted from the 23-item validated instrument used by Kotecki, Elanjian & Torabi [10] with a slight modification done by merging two items on tobacco into one.

A total of 19 of the 22 health promotion behaviours dealt with recommendations from the US government health agencies, these included tobacco and alcohol use, physical activity, diet, injuries, sexual behaviour, stress, and preventive services. The remaining three items dealt with pharmacists' dispensing role that could prevent drug therapy problems.

The third part of the survey instrument listed the same 22 items and asked for their perceived level of involvement using a five-point Likert scale (very uninvolved, uninvolved, uncertain, involved, very involved).

The final aspect of the study asked pharmacists of their perceived barriers to integrating health promotion activities into their daily practice and they were asked whether they would be willing to participate in continuing education courses to learn more about health promotion.

Method of Data Collection

Community pharmacies were visited based on the directory collected from the Pharmacists Council of Nigeria. At the premises, the purpose of the study was explained to the Pharmacist and the questionnaires were served.

Method of Data Analysis

Descriptive analysis of the data obtained was done for the demographic details, charts and tables were used to express the percentages of different options under each health-related item. The average score for each health related behaviour was obtained for both variables (importance attached and involvement) and Pearson's correlation coefficient was used to establish the association between the two variables

RESULTS

Response rate & Demographics

A total of 150 questionnaires were returned filled and useable giving a response rate of 80.65% (150/186).

The gender distribution of the respondents was close to equal. 51.3% (77) of the respondents were males and 48.7% (73) were females. About two-thirds of the respondents were employees (95, 63.3%) and the remaining one-third were owners (55, 36.7%)**.** Also, 36.7% of the respondents have had more than 5 years of community practice experience while 24% had less than one year of experience in community practice (Table 1.0).

Perception of Health Related Behaviours

The three medication-related behaviours received the highest percentage of "very important" ratings. These were; take prescription drugs as prescribed, take OTC medications as directed, and be knowledgeable about drug contents and side effects with 91.3%, 70% and 62% respectively.

Out of the 19 non medication-related behaviours, the use of condoms ranked highest with 66.7%, followed by maintaining normal blood pressure (59.1%), elimination of tobacco product (58.7%), use of seat belts (57.3%). Others with above 50% response of "very important" were; eat variety of foods (balanced diet), balance food one eats with physical activity, avoid undue stress. The remaining 12 items received less than 50% response as "very important" (Fig.1.0).

The three medication-related behaviours received over 50% of respondents claiming to be "very involved" (Fig. 2.0).

The mean score of the health related items for perception and involvement is shown in Table 2.0. The mean score for the level of importance attached and degree of involvement were 4.35 and 4.0 respectively on a scale of 5.0. Pearson product-moment correlation showed there was a strong, positive correlation which was statistically significant ($r = .869$, $n = 22$, $p < .0005$)

Impatience on client's part and time constraints are the two major barriers highlighted by over 60% of the respondents.

List of Health Related Behaviours in Figures 1.0 & 2.0 (1. Take prescription drugs as prescribed**, 2.** Take OTC medications as directed**, 3.** Be knowledgeable about drug contents and side effects**, 4.** Maintain normal blood pressure**, 5.** Maintain normal blood cholesterol level**, 6.** Maintain ideal weight**, 7.** Choose a diet low in fat, saturated fats and cholesterol**, 8.** Choose a diet moderate in salt, 9. Choose a diet moderate in sugar, **10.** Choose a diet with plenty of grain products, vegetables and fruits**, 11.** Balance the food one eats with physical activity to maintain weight**, 12.** Eat variety of foods i.e. balanced diet**, 13.** If one drinks alcohol, do so in moderation**, 14.** Elimination of all tobacco products (cigarettes, pipe smoking etc)**, 15.** Avoid undue stress**, 16.** Live in a safe neighbourhood**, 17.** Have an annual physical examination for preventive screening**, 18.** Always use seat belts when driving**, 19.** Always use condoms if one has multiple sex partners**, 20.Engage** in aerobic activity three or more times a week, 21. Engage in strength training of moderate intensity of at least twice a week**, 22.** Regularly practicing relaxation techniques (yoga, massage).

DISCUSSION

Health Promotion spans the length of all human activities, it has a broad coverage and so are the practices; from safety precautions like fastening of seat belt, to habitual changes like tobacco cessation, they are all regarded as health promotion practices. This broad coverage leaves the possibility of neglecting some aspects of health promotion if perceived as not so important.
The level of importance attached to a particular health behaviour is expected to affect the level of involvement of an individual in promoting the value. It has been noted that "Understanding the importance pharmacists attach to vital health promotion behaviours provides information about what pharmacists may emphasize in assessing the behaviours of their patients and providing health education." [10]

The three medication-related behaviours got the attention of majority of the pharmacists as "very important" to health promotion; 91%, 70% and 62%. This is not surprising as Pharmacists see themselves first as custodians of drugs and may want to see other activities as an extended role. The fact that these medication-related behaviours got the highest response rate of very important is consistent with other studies done in this regard. (Kotecki, Elanjian & Torabi 2000; Oparah & Okojie 2005). It also concurs with Eades, Ferguson & Caroll, assertion that, most pharmacists viewed public health services as important and part of their role but secondary to medicine related roles. [15]

In their perception of the remaining personal health-related behaviours, only 7 out of the 19 behaviours received a "very important" vote from more than 50% of the respondents. A similar study carried out in Benin, Nigeria found 9 out of 20 behaviours with more than 50% "very important" response while the study done in Indiana, US, reported 10 out 20 behaviours. It is noteworthy however, that, there are four behaviours that enjoyed a favourable disposition to their promotion in the two similar studies considered as well as in this study. These are; use of condoms if one has multiple sex partners, maintaining normal blood pressure, elimination of all tobacco products and balancing the food one eats with physical activity to maintain or improve weight. [10, 11]

Also worthy of note are the three health related behaviours that are consistently among the least rated as very important in previous studies done as well as in this study. These are; engaging in aerobic activity three or more times a week, engaging in strength training of moderate intensity of at least twice a week and regularly practicing relaxation techniques such as massage, meditation etc. The average mean score for the level of importance attached and degree of involvement were 4.35 and 4.00 respectively on a scale of 5.0. A trend shown in this study hints

at a positive correlation between the level of importance attached and the perceived degree of involvement observed. A Pearson product-moment correlation showed there was a strong, positive correlation which was statistically significant ($r = .869$, $n = 22$, $p < .0005$).This suggests a very strong and positive relationship between pharmacists' perception and involvement.

Impatience on the part of clients (67.3%) and time constraints (68.0%) were the highest identified barriers to health promoting activities. Though inadequate number of staff received a low response of 18.7%, adequate staffing would free up more time for the pharmacists to carry out health promotion activities, but how many workers could be said to be adequate is a question begging for an answer. Considering the fact that there is usually no extra pay for these activities, owner of stores would naturally be reluctant to employ more workers for activities that yields no further profit. Also of note in the study, is the revelation that majority of our respondents do not see poor remunerations, lack of reimbursements and even inadequate training as barriers to health promotion activities.

Almost all our respondents (99%) indicated the willingness to learn more about health promotion through continuing education. Relevant authorities are thus advised to introduce a health promotion course in our continuing education curriculum.

CONCLUSION

The level of importance attached to promoting health-related behaviours among community Pharmacists in Lagos is high and there is a strong positive relationship between the level of importance attached to a particular behaviour and the degree of involvement in promoting it. Community Pharmacists in Lagos State should be encouraged to be involved in health promotion practices to boost health services in the State.

Health promotion roles should be introduced early enough in pharmacy curriculum in order to sensitize pharmacists of their very important health promoting roles.

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Table 1: Demographic distribution of respondents

		Percent (%)
Gender of Respondents	**Frequency**	
Male		51.3
Female	77	48.7
Total	73	100
Years of Community Pharmacy Practice	150	
Less than 1 year		24.0
1-5 years	36	39.3
6-10 years	59	19.3
11-15 years	29	7.3
More than 15 years	11	10.0
Total	15	100
Ownership status Amongst Respondents	150	
Owner		36.7
Employee	55	63.3
Total	95	100
	150	

Table 2: Health-Related Behaviours' Mean score for level of Importance and Degree of Involvement for all Respondents

HEALTH-RELATED BEHAVIOURS	Mean Score (SD) Importance	Mean Score (SD) Involvement
Take prescription drugs as prescribed	4.90 ±0.34	4.43 ±1.05
Take OTC medications as directed	4.67 ±0.54	4.33 ±1.03
Be knowledgeable about drug contents and side effects	4.58 ±0.58	4.31 ±1.05
Maintain normal blood pressure	4.56 ±0.56	4.27 ±1.04
Maintain normal blood cholesterol level	4.43 ±0.67	3.94 ±1.12
Maintain ideal weight	4.34 ±0.63	3.95 ±1.08
Choose a diet low in fat, saturated fats and cholesterol	4.26 ±0.69	4.00 ±1.06
Choose a diet moderate in salt	4.36 ±0.61	4.01 ±0.98
Choose a diet moderate in sugar	4.37 ±0.63	4.00 ±1.06
Choose a diet with plenty of grain products, vegetables and fruits	4.37 ±0.71	4.07 ±1.05
Balance the food one eats with physical activity to maintain weight	4.50 ±0.62	3.96 ±1.05
Eat variety of foods i.e. balanced diet	4.67 ±0.73	4.04 ±1.00
If one drinks alcohol, do so in moderation	4.39 ±0.68	3.81 ±1.15
Elimination of all tobacco products (cigarettes, pipe smoking etc)	4.41 ±0.84	3.91 ±1.21
Avoid undue stress	4.44 ±0.68	3.87 ±1.09
Live in a safe neighbourhood	4.30 ±0.74	3.66 ±1.11
Have an annual physical examination for preventive screening	4.29 ±0.77	3.65 ±1.14
Always use seat belts when driving	4.55 ±0.54	3.70 ±1.27
Always use condoms if one has multiple sex partners	4.57 ±0.73	3.86 ±1.24
Engage in aerobic activity three or more times a week.	3.91 ±0.91	3.65 ±1.11
Engage in strength training of moderate intensity of at least twice a week	3.58 ±0.99	3.11 ±1.14
Regularly practicing relaxation techniques **(yoga, massage)**	3.49 ±1.02	3.24 ±1.57
MEAN SCORE (n=150)	**4.35**	**4.00**

SCORE CHART: Very unimportant=1, Unimportant =2, Uncertain=3, Important=4, very important=5
Very uninvolved=1, Uninvolved=2, Uncertain=3, Involved=4, Very involved =5

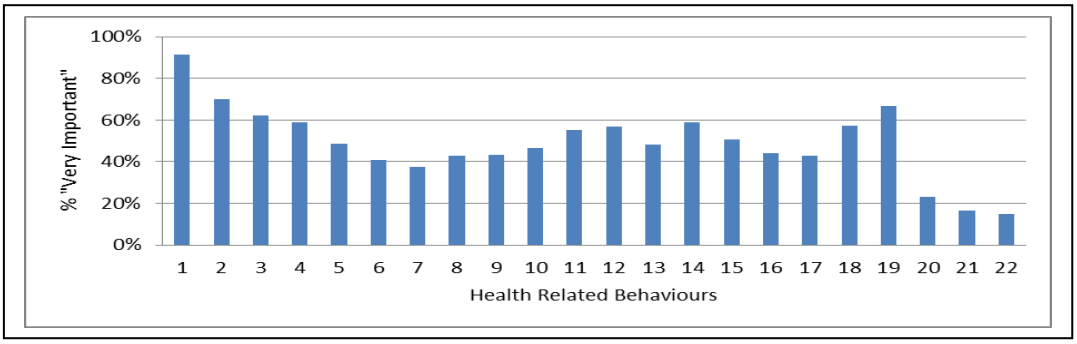

Figure 1.0: A bar chart showing the percent response each health related item received as "very important"

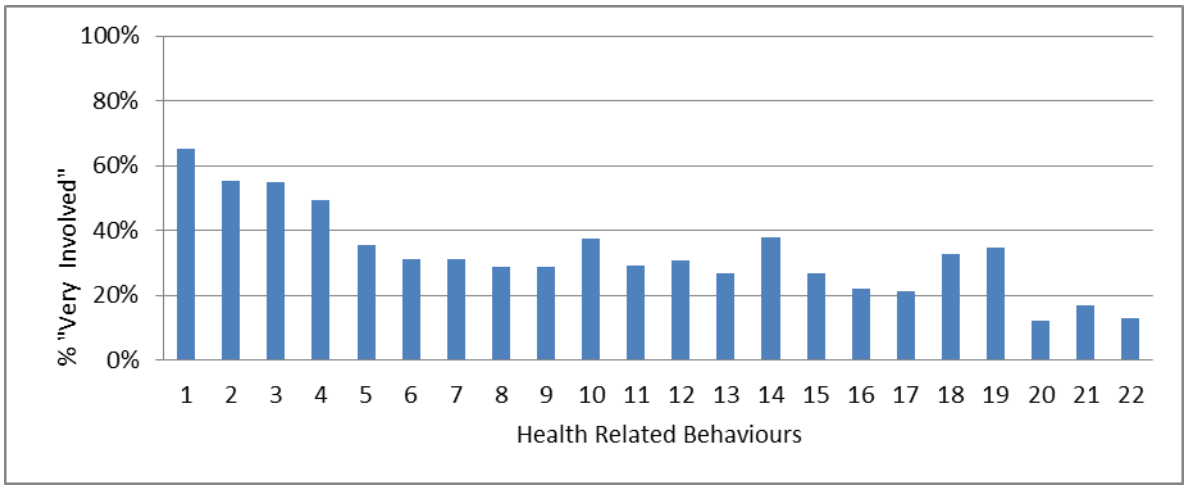

Figure 2.0: A bar chart showing the percent response each health related item received as "very involved"

PLANNING AND IMPLEMENTING PHARMACEUTICAL CARE FOR PEOPLE LIVING WITH DIABETES (PLWD) IN A COMMUNITY PHARMACY

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ABSTRACT
Diabetes refers to a group of complex endocrine disorder and is known as hazardously raised level of blood glucose (sugar). It is a chronic and incurable condition that can be well managed. This study aimed to plan and implement Pharmaceutical Care (PC) to People Living with Diabetes (PLWD) by Pharmacists as part of cognitive services in the community and to determine its outcome using Mainland Local Government Area of Lagos State as a Pilot Study. PC is a patient-centred, outcome-oriented pharmacy practice. It is an innovative way of practising pharmacy that has the potential to make drug-therapy and disease management safer, more effective and more convenient for the patient. One hundred and twenty (120) patients were enrolled based on the sample size calculation (using Fischer's formula) and inclusion criteria, five Pharmacists were also enrolled for this study. One community pharmacy-Pillbox Pharmacy, served as the health station. Self-administered questionnaire were pre-tested and administered to the patient and a separate one to the Pharmacist. The subjects were educated on diabetes management and PC prior to the intervention, after which patients were assigned to the pharmacist. The pharmacist provided PC to patient at no charge for four months, using our systematic approach to the delivery of PC. This approach included development and use of various documentations such as patients database file, record cards, etc. Patient education and monitoring of patient vitals such as blood pressure, fasting plasma glucose were done by the Pharmacists. The paired t-test was used to analyse the results obtained for significant statistical difference before and after the intervention. The results obtained showed humanistic, clinical and economic outcomes.
Humanistic outcome: there was improvement in patient knowledge of diabetes and its management from 40% good to 90% good. Patient satisfaction with treatment was increased from 40% to 85%. **Economic outcome**: there was a decrease in hospitalization and increased patient productivity; patients' productivity at work increased from 78% to 86%. **Clinical outcome**: Eighty per cent (80%) had their fasting plasma glucose (FPG) within normal clinical range (70 to 126mg/dl). Using the paired t-test, the difference in the mean of patients FPG before and after the intervention was 1.325 ± 0.76(SD).$P<0.05$. Hence, there was a statistical significant difference in patients FPG before and after intervention. There was a statistical significant difference in patients' blood pressure value before and after the intervention; mean BP (i.e. before – after) was 0.650 ± 0.48 (SD), $p< 0.05$. This study has shown that it is feasible to plan and implement PC in the community. It also showed that FPG and BP values were significantly improved after implementing PC service.

Keywords: *Diabetes, Fasting Plasma Glucose, Pharmaceutical care.*

INTRODUCTION

Diabetes refers to a group of complex endocrine disorder and is known as hazardously raised level of blood glucose (sugar).Glucose is vital to health because it is the main source of energy for cells that makes up muscles and tissues.

According to International Diabetes Federation (IDF), more than 230 million people worldwide are living with Diabetes [1]. Seven out of ten countries with the highest number of people living with diabetes are in developing countries.
In 2004, an estimated 3.4 million people died from consequences of high fasting plasma glucose [2]. More than 80% of diabetes deaths occur in low and middle-income countries [3].
WHO projects that diabetes will be the 7th leading cause of death in 2030 [4].

Pharmaceutical care (PC) is the responsible provision of drug therapy for the purpose of achieving definite outcome that improves a patient's quality of life [5].
It is "a practise in which the practitioner takes responsibility for a patient's drug related needs and holds his/her self responsible for meeting these needs"[6].
PC is a patient-centred, outcome-oriented pharmacy practice that requires the Pharmacist in co-operation with the Patient and other members of the healthcare team to design, implement and monitor a pharmaceutical care plan that will provide the stated therapeutic outcomes. PC addresses the patient's drug-related needs comprehensively through a scheduled outline of tasks, in which the practitioner makes sure that the drug therapy is appropriately indicated, effective, safe and convenient. These include detecting prescribing and medication errors, involving patients in decision making, improving adherence and reducing waste of scarce resources. The target of PC is to maintain the patient at the highest possible level of functional and psychological well-being through optimal management of drug therapy.

Studies have shown that the adoption of PC services resulted in cost savings for health care:
In the Minnesota project, Strand documented that almost half of the patients had drug therapy problem that needed to be resolved. The most common problem was found to be patients not receiving drug therapy that was needed and adverse drug reactions. On this project, the cost benefit ratio for PC practice based on 1000 elderly patient was found to be 11:1. This is one of the highest cost-benefit ratios found in literature.[7]

Other benefits of PC include:
It improves patient knowledge of the disease and its management.
It provides an improved platform for medication history and documentation, etc.
Diabetes is a chronic and incurable condition. It needs more Pharmacist involvement because of the increasing number of people affected by the disease and this portends an increase morbidity and mortality as well as increase in the amount of money directly or indirectly spent on the disease and its complications. This study aimed to plan and implement Pharmaceutical Care (PC) to People Living with Diabetes (PLWD) by Pharmacists as part of cognitive services in the community and to determine its outcome using Mainland Local Government Area of Lagos State as a Pilot Study.

METHODS

Study Area

This research was conducted in Mainland local Government area of Lagos state. According to Association of Community Pharmacist of Nigeria (ACPN) directory, the zone has about 55 registered pharmacies.

Study Design

This study is a retrospective and prospective study involving the use of pre-tested self-administered questionnaire amongst pharmacist and patient.

Study Period
The study period was for seven months i.e. from January 2012 to August 2012.
Sampling Technique
Convenience sampling method was used.

Study Population
This comprised of adult type 2 diabetic patient that are interested in PC

Inclusion Criteria
- Patient with type 2 DM
- Patient who are forty years old and above
- Patient with reasonable geographic access to the pharmacy.

Procedure
One community pharmacy-Pillbox Pharmacy; served as the health station. Based on the sample size calculation (using Fischer's formula) and inclusion criteria, one hundred and twenty (120) patients were enrolled for this study. Five pharmacists and two pharmacists assistant participated in this study as well.
Self-administered questionnaire were designed and pre-tested before administering to the patient and a separate one to the Pharmacist via the selected community Pharmacy.
Pharmacists that participated in this project were enrolled for a full day workshop on diabetes management and were assigned to the patient. Once assigned, the pharmacist contacted the patient to set up appointment and schedule visits regularly. They provided PC at no charge for four months using the systematic approach to the delivery of PC i.e.

Assessing and Identifying Drug Therapy Problems
Here a good communication was established with the patient, carer and some members of the health care team in order to synthesize and interpret relevant information.
The Pharmacist collected the subjective and objective assessment information and appropriate documentation was made. The IDEAL method i.e. Identification, Definition, Exploration, Action, Looking and Learning was employed.

Set PC Goals
For this study, controlling blood sugar (glucose) level was the priority; to make patient feel better and to prevent long-term complication. Our set goals were based on the cornerstones to controlling diabetes, which included five basic steps:
- Monitoring blood glucose and blood pressure levels- regular Fasting Plasma Glucose test and blood pressure check were done working towards approaching normal values; FPG<126mg/dl and BP<130/80.
- Nutritional recommendation- i.e. varied and healthy diet, stressing glycemic index and caloric calculation.
- Staying active
- Maintaining a healthy weight
- Using medication appropriately.

Development of a Care Plan i.e. PC for PLWD
The set goals were used to develop a care plan and patient were informed about the general content of the care plan as means of gaining their agreement regarding the therapy. The step for our care plan includ00es:
- Creating a comprehensive patient database.

- Assessing for actual and potential drug related problems.
- Establishing therapeutic goals.
- Monitoring parameters with end points.
- Documenting the patients' progress towards therapeutic goals.

Implementing The Care Plan

This was based on the agreement with the patient and/or caregiver. The Pharmacist provided integrated and quality PC to the Patient. Pharmacist intervention included:
a- Patient focused
b- Drug focused.

Patient focused intervention includes:
Health promotion- educating and informing patient about ways in which their behaviours and lifestyle affects their diabetes. Patients were informed on preventive measures to avoid ill-health and diabetes complication and were encouraged to adopt healthy behaviours.
Empowerment- i.e. individuals are to take charge of their health and actions to control modifiable determinants of health. These include:

- ❖ Self-care e.g. foot care.
- ❖ Self-monitoring i.e. self-monitoring of blood glucose (SMBG) done at the pharmacy.

The drug focused interventions were also included in the study.
Regular appointments were made with the patient.

Monitoring and Evaluation

The expected outcomes were evaluated in relation to the therapeutic objectives to deter mine whether optimum PC has been provided and drug therapy problem has been resolved. The expected outcomes were:

A. **Clinical outcome** which included:
   i. Glycemic control: assessed by FPG<126mg/dl
   ii. Blood pressure < 130/80 mmHg
   iii. Symptom control: polyuria, polyphagia, polydipsia.

B. **Humanistic outcomes**:
   i. Patients knowledge of diabetes and its management
   ii. Health related quality of life
   iii. Patients' satisfaction with treatment.

C. **Economic outcomes**:
   i. Increased productivity due to reduced sick time.
   ii. Reduction in hospitalization and length of stay etc.

Individualized monitoring strategies were identified and developed.
The Pharmacists regularly fills various documentation used such as health check profile book, Patients record card, Pharmacists intervention forms etc.
The Pharmacist reviews on-going progress in achieving desired outcome, with the patient regularly.
Finally, a report was made by the Pharmacist to the health station and communicated to the patient and other healthcare provider as appropriate.

Ethical Consideration

An introductory letter was obtained from the West African Postgraduate College of Pharmacists (WAPCP) to the chief Pharmacist of the selected community Pharmacy.
Subjects who consented to the study were assured of confidentiality of their data.

RESULTS

The results obtained showed humanistic, clinical and economic outcomes.

Humanistic outcome: There was improvement in patient knowledge of diabetes and its management. Patient attitude and satisfaction with treatment was increased from 40% to 85%.

DISCUSSION

Before the intervention, more than half (60%) were satisfied with the treatment they received in relation to their disease. After the intervention, 85% were satisfied while 15% were not. 15 % is sizeable and should encourage the participant pharmacist and in general pharmacist to inculcate PC in their daily practice.

PLWD enrolled for this study demonstrated improved humanistic, economic and clinical outcomes.

Humanistic outcome: There was improvement in patient knowledge of diabetes and its management. Patient attitude and satisfaction with treatment was increased from 40% to 85%.

Economic outcome: there was increased patient productivity.

Clinical outcome: Eighty per cent (80%) had their fasting plasma glucose level within normal clinical range (70 to 126mg/dl).

This was in line with Ashville project done in America: the Patients enrolled in the study had significant improvement in clinical indicators of diabetes management and employers experienced a decline in mean total direct medical costs. [8].

It is also in line with key findings of 'PC in community Pharmacies: Practice and Research in Sweden' [9].

A collaborative approach involving the patient, physician and diabetes educator (pharmacists, nurses, etc.) can lead to lowering of patient's blood pressure, blood sugar (close to normal), decrease hospitalization and improved quality of life for PLWD[1]

Limitation of The Study

These include:

- Time frame- an effective PC and adequate intervention is very time consuming.
- Limited number of personnel.
- Space – insufficient counselling room.

CONCLUSION

This study has shown that it is feasible to plan and implement PC in the community. It showed that FPG and BP values were significantly improved after implementing PC service. The patients demonstrated improvement in:

- health status of majority of the patient,
- pharmacist and patient knowledge of diabetes and its management,
- patient attitude and satisfaction with therapy

Effective action to reduce the threat of diabetes therefore remains an urgent priority across the globe, hence, there is need to properly plan and implement PC for PLWD. From the perspective of this study, it is also relevant that professions such as pharmacy are adapting to meet twenty-first century health care needs.

Pharmacists should begin to focus more of their working effort on supporting and caring for chronic disease and long term conditions like diabetes, hypertension etc. and enhance wider health promotion activities.

ACKNOWLEDGEMENTS

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We appreciate the five Pharmacists, two Pharmacists Assistant and the staffs of Pillbox Pharmacy that participated in this project.

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TABLE 1: Mean Difference Of Intervention Before And After The Study

Vital signs	Mean difference	Standard deviation	t-value	P-value
Fasting plasma glucose [FPG]	1.325	0.758	19.155	0.000
Blood pressure[BP] value	0.650	0.479	14.866	0.000

Table legend: $p<0.05$ shows statistical significance.

Using the paired t-test, there was a statistical significant difference in patient diabetes state, Mean FPG (i.e. before – after intervention) was 1.325 ± 0.76 (SD), $p < 0.05$.

The paired t-test also showed a statistical significant difference in Patients' blood pressure value before and after the intervention, Mean BP (before- after intervention) = 0.650 ± 0.48 as $p<0.05$

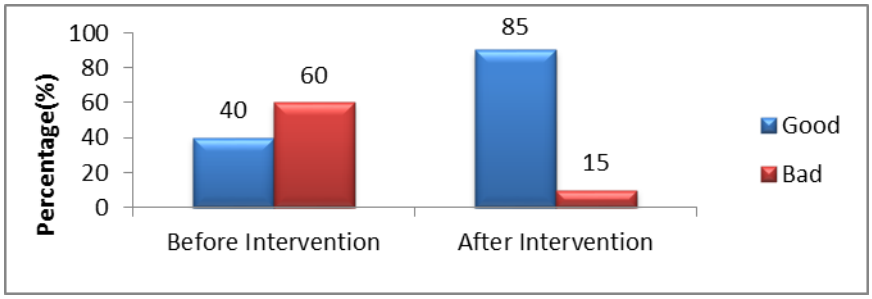

Figure A: Patients Knowledge of Diabetes Mellitus Management

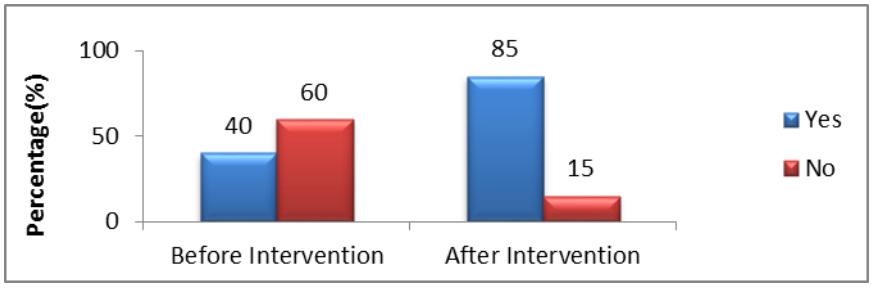

Figure B: patients' satisfaction with management

Economic outcome: there was increased patient productivity.

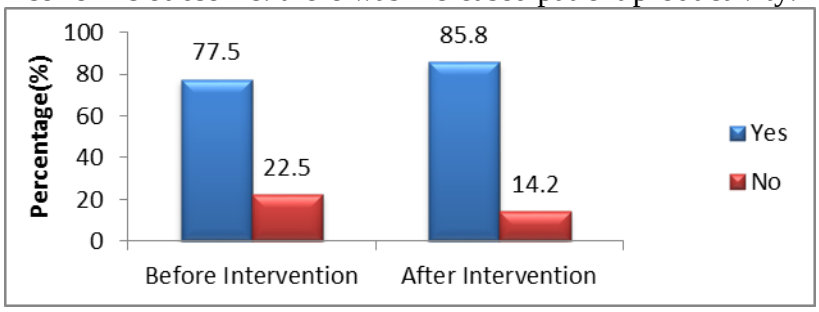

Figure C: Patients productivity at work

Clinical outcome: Eighty per cent (80%) had their fasting plasma glucose level within normal clinical range (70 to 126mg/dl).

PREPONDERANCE OF PALMITOLEIC ACID IN *MORINGA OLEIFERA* (Lam.) SEEDS FROM GAS CHROMATOGRAPHIC ANALYSIS

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ABSTRACT
Moringa oleifera is the most widespread species of the genus *Moringa*, the only genus in the family Moringaceae. It is a very popular plant used in traditional herbal medicine. Different parts of the plant contain a profile of important minerals and phytochemicals. The leaves and seeds are good sources of proteins, vitamins, beta-carotene, amino acids, antioxidants and phenolic compounds. Dried plant parts were pulverized and subjected to proximate analysis while the oils were extracted from the seeds with hexane using sohxlet apparatus and analyzed with gas chromatography. The chemical contents of *M. oleifera* leaves obtained through proximate analysis showed it had more protein than the seeds containing 45.28% protein while the seeds had 40.10%. Fat contents of the seeds were 3.09% while the leaves had 6.40% fat. Gas chromatographic analysis of the oils showed the presence of various fatty acids and other organic compounds with palmitoleic acid being the most abundant with 48.41% yield of total oils and oleic acid being 11.45% much less than earlier reported. Palmitoleic acid has shown possible influence in fatty liver deposition/production, insulin action and fatty acid synthase. This makes *M. oleifera* seeds very important new source of natural therapy for hyperglycemia and hypertriglyceridemia.

Keywords*: Moringa oleifera, gas chromatography, proximate analysis, palmitoleic acid*

INTRODUCTION

Oils in plants are usually classified in two ways; fixed oils e.g. Shea butter, olive oil and essential or volatile oils e.g. citrus oil, mint oil. They are used extensively in herbal medicine practice and serve diverse purposes in amelioration of health and can be applied to a wide spectrum of diseases. Oils also find application in the flavour and fragrance industry, pharmaceutical industry, and in aromatherapy. They can also function as antimicrobials (Ogbolu *et al*., 2007, Warnke *et al*., 2009). Essential oils are usually obtained by steam or hydro distillation of botanicals while fixed oils are obtained by defatting procedures. Extracts from hexane and other hydrophobic solvent are called *concretes*, which are a mixture of essential oil, waxes, resins, and other lipophilic (oil soluble) plant material.

Different parts of plants can yield oils, including the flowers, leaves, seeds, roots, stems, bark, and wood. The oil of the same plant can vary strongly in composition depending on the species, location, soil and weather conditions, and level of expertise and care given by farmers and distillers. For such reasons, the characterization of the oils through chemical analysis is a mandatory step in the production chain, to be carried out by both researchers and quality control labs. Practically all oils consist of chemical mixtures that are often quite complex; they vary widely in chemical composition. Almost any type of organic compound may be found in oils (hydrocarbons, alcohols, ketones, aldehydes, ethers, oxides, esters, and others). Plants of the same species grown in different parts of the world usually have the same components, but the quantities of these components may differ. Certain physical constants of oils are significant: specific gravity, refractive index and sometimes optical rotation and solubility in alcohols.

Interest in the oil extracted from *Moringa oleifera*, (Fig.1 1nd 2) known commercially as 'Ben' or 'Behen' oil, has existed for well over a century. The first recorded study of the composition of the oil was carried out in 1848 which revealed a fatty acid with a high melting point (Anon 1904). This was subsequently called behenic acid from which the commercial name for *M. oleifera oil* came (Anon, 1904) *M. oleifera* is the most common of the genus and considered a multipurpose tree native to the foothills of the Himalayas in northwestern India (Olson, 2010) and cultivated throughout the tropics (Janick and Paull, 2008). Schill, 2008 reported high content of oleic acid in *M. oleifera*. It is considered a potential oilseed feedstock for biodiesel (Schill, 2008). *M. oleifera* leaves and seeds are greatly popular for management of many and diverse health problems, spanning from anaemia to high blood pressure.

Our interest of study is the mineral constituents of leaves and oils of seeds of *M. oleifera* obtained from Ikorodu, Lagos State. Nigeria

METHODS

Extraction of *M. oleifera* seed oil.

Solvent extraction

M. oleifera seeds were collected from Ikorodu area in Lagos state 6.6000° N, 3.5000° E and 86% humidity. They were dried in an oven at 40-50^{o}C and dehusked. The dehusked seeds were weighed and 230g of the seed were pulverized to coarse powder. The seed powder was transferred into a glass jar and soaked in petroleum-ether for about 72 hours for extraction of the oil from the seed. Further extraction was carried out using the Soxhlet apparatus with the same solvent for about 4 hours. The oil extract was collected in a beaker and exposed to dry air at room temperature to ensure the solvent evaporates completely and then stored in a dark bottle till required.

Proximate analysis

Carbohydrate content

Carbohydrate content was determined using the Anthrone method (Trevelyan, 1952). For each of the samples, 0.1g was hydrolysed by keeping in a boiling water bath for three hours with 5ml of 2.5N HCl and cooled to room temperature. Then neutralized with solid sodium carbonate until effervescence ceases. The volume was then made up to 100ml and centrifuged. The supernatant was collected, and 0.5 and 1ml were taken for aliquot analysis. The standards were then prepared by taking 0.2ml, 0.4ml, 0.6ml, 0.8ml and 1ml of the working standard. The volumes were then made up to 1ml by adding distilled water. 4ml of anthrone reagent was added. Heating on the water bath went on for eight minutes, after which it was left to cool. The absorbance was read at 630nm using a spectrophotometer.

A standard graph was drawn by plotting concentration of the standard on the X- axis versus absorbance on the Y- axis. From the graph, the amount of carbohydrate present in the sample was calculated using the following equation

$$100\text{mg of the sample} = \frac{\text{mg of glucose X 100}}{\text{Volume of test sample}}$$

Total protein

0.1g of the sample was transferred into a test tube, and homogenized with 10ml of distilled water using a mortar and pestle. The homogenate was centrifuged for 5 minutes at 300rpm. To the supernatant (1ml), 5ml of alkaline copper reagent was added and was allowed to stand for about 20 minutes at room temperature. Folin C reagent of 0.5ml was added and shaken for about 10 minutes, and absorbance was taken at 570nm.

Lipids content
The sample (1g) was added to 10ml of chloroform-methanol mixture of (2:1), centrifuged and the supernatant collected. 5ml of normal saline was added with continuous shaking. The chloroform layer was collected into a beaker, and then heated on a water bath until all the chloroform evaporates. The resulting extract was allowed to cool. Then the quantity was calculated thus

$$\text{Percentage of lipids} = \frac{W3 - W1}{W2 - W2} \times 100$$

W3 = Lipids + beaker
W2 = sample + beaker
W1 = empty beaker

Moisture content
The sample was placed in an empty dish, and the weight of the sample and the dish was obtained. The weight of the dish alone was also determined. The sample in the dish was placed in the oven at 105°C, for a minimum of 4 hours. The weight of the sample in the crucible was checked at intervals, until constant weight was achieved.

$$\text{Moisture content} = 100 - \frac{(W3 - W1 \times 100)}{W2 - W1}$$

W3 = final weight of sample and dish
W2 = initial weight of sample and dish
W1 = weight of dish

Ash content
The sample in the dish was placed in a furnace, and maintained at 560 – 600 °C. The ash was thus determined using the calculation below;

$$\text{Ash content} = \frac{W3 - W1 \times 100}{W2 - W1}$$

W3 = weight of crucible and ash
W2 = weight of crucible and sample
W1 = weight of crucible

Crude fibre
The crude fibre was determined by adding up the percentage of the crude protein, carbohydrate, lipid, ash, and moisture content, and then subtracting the summation from 100.

Phytochemical tests
Tests for tannins, glycosides, anthraqinones, saponins, alkaloids, sterols and triterpenes were carried out according to Harbone, 1998.

Gas chromatography
Using helium as the carrier gas, the components were separated on 30 m x 0.25 mm i.d., 0. 25 µm film thickness. The injector temperature was set at 250 ºC. The column was initially maintained at 50 ºC for 5 min; subsequently the temperature was increased to 210 ºC at a rate of 5 ºC/min and finally held for 5 min FID Detector temperature was set at 270 ºC.

hypodermic needle. The sample port was maintained at 270°c so as to vaporize the sample, and allow the sample pass through the column. The sample was allowed to run in the column for 45 minutes, at an average velocity of 69.246cm/seconds and pressure of 12psi. During this period which the sample runs through the column, the various components were separated which is indicated by the various peaks, indicated by the detector.

Refractive index

The surfaces of the lower and upper prism were first wiped with methanol to remove impurities which may interfere with the reading. The oil sample was mounted on the lower prism. The dark region and the light region were examined using the eye piece. The adjustment knob was adjusted so that the intersection between the two bands coincide with the point of intersection of the 2 cross lines seen under the eye piece.

RESULTS

Figure 1. Life form picture of *M. oleifera* plant in a garden

Figure 2. Life form picture of *M. oleifera* seeds

Table 1 Gas Chromatogram of *M. oleifera* seed oil

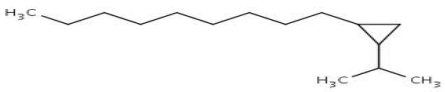
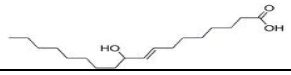
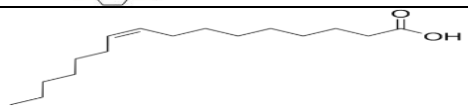
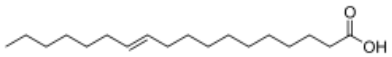
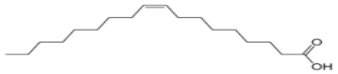
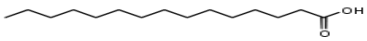
Peak Number	Retention Time	Percentage of Total	Compound
1	13.516	4.495	1-Isopropyl-2-nonyl-cyclopropane 
2	27.857	16.209	n-hexadecanoic acid/ Palmitic acid 
3	30.095	0.677	8-octadecenoic acid 
4	31.577	48.401	 9-hexadecenoic acid/Palmitoleic acid
5	31.613	5.815	Cis-vaccenic acid 
6	31.645	11.450	6-0ctadecenoic acid/Oleic acid 
7	31.878	5.815	Pentadecenoic acid 
8	36.975	2.236	9-octa decenal 
9	38.166	2.544	Tetracontane 
10	41.741	0.847	Octadecamethyl-hexsiloxane
11	44.099	1.481	Pentadecyl, methoxyacetic acid

Table 2. Proximate analysis of components of *M. oleifera* seed

Proximate analysis	Average (%)
Carbohydrate	31.63±0.09
Protein	40.10±0.05
Crude fat	3.09±0.00
Moisture content	9.42±0.00
Ash value	6.19±0.17
Crude fibre	9.57±0.16

Table 3. Proximate analysis of *M. oleifera* leaf

Proximate analysis	Average (%)/SD
Carbohydrate	18.92±0.10
Protein	45.28±0.02
Crude fat	6.40±0.01
Moisture content	12.76±0.00
Ash value	10.63±0.01
Crude fibre	6.00±0.04

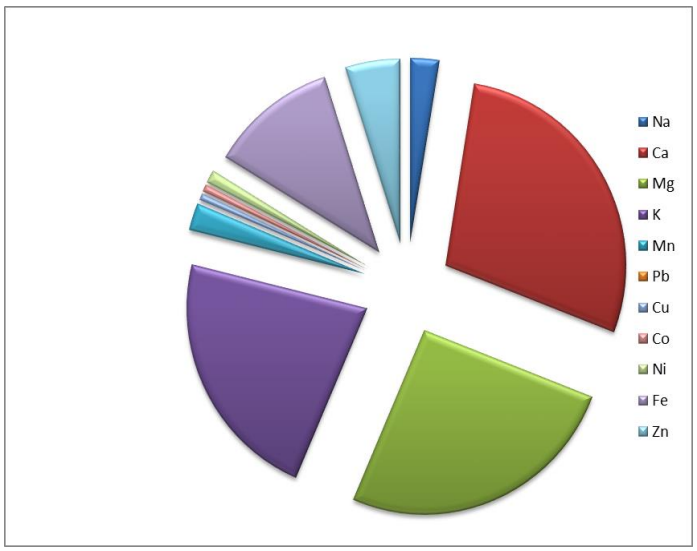


Figure 3. Mineral contents of *M. oleifera*

DISCUSSION

Previous studies on *Moringa oleifera* species found an abundance of oleic acid and probably lead to the specific name of *oleifera*. The seeds used for these studies showed a preponderance of palmitoleic acid (Table 1.) also known as 9-hexadecenoic acid, and is an omega-7 monounsaturated fatty acid. It is known to be biosynthesized from palmitic acid by the action of the enzyme delta-9 denaturase. It has also been shown to increase insulin sensitivity by suppressing inflammation, as well as inhibit the destruction of insulin-secreting pancreatic beta cells (Yang *et al*., 2011). This may be the reason for the success of the seeds in lowering cholesterol levels in consumers.

The proximate analysis (Table 2 and 3) showed that the seed is rich in carbohydrate and protein. This is contrary to the result obtained by Nzikou *et al.*, 2009 which showed a higher percentage of crude fat. This variation of result could be due to variation in climate of the various geographical locations from which the pods were harvested, as the *M. oleifera* species used for

the analysis of Nkizou *et al*., was obtained from Brazzaville whereas the species used for this analysis was obtained from Ikorodu area of Lagos, Nigeria. It is equally of interest to note that the most prevalent mineral elements in *M. oleifera* are magnesium and calcium (Fig. 3) which were found to be 49.50 and 54.85 (mg/100g) in the seed, 42.80 and 54.95(mg/100g) in the leaves respectively. Magnesium plays a major role in photosynthesis, carbohydrate metabolism, nucleic acids metabolism etc. (Russel, 1973). The presence of calcium in high concentrations therefore makes *M. oleifera* a good dietary source of the mineral for patients with calcium deficiency.
The presence of flavonoids can be used to explain the anti-inflammatory, anti-oxidant and hypoglycaemic effects of these seeds.

Oils have many properties which are used for their identification. One of such property is the refractive index. The refractive index of *M. oleifera* seed oil was carried out, and compared with the reference value. The refractive index was found to be 1.470 using the Abbe refractometer at room temperature of 25°c, while the reference value was found to be 1.4671 at 20°c. The slight difference in the value obtained compared to the reference might be due to the variation in the sensitivity of the refractometers, as well as slight variation the temperature at which the readings were taken.

The density of the *M. oleifera* oil was also ascertained and was found to be 0.893g/ml, the reference value was found to be 0.897g/ml. the variation could also be due to the variation in temperature at which the experiment was carried out. This study has confirmed again the dietary importance of *M. oleifera* and added to existing knowledge that some species of the plant have a preponderance of palmitoleic acid a highly useful compound for carbohydrate metabolism.

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PHYTOCHEMICAL AND MICROBIOLOGICAL EVALUATION OF LEAVES AND FLOWERS OF *CRATERISPERMUNM LAURINUM POIR (BENTH)* (RUBIACEAE)

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Antibiotic resistance is a serious and growing phenomenon in contemporary medicine and has emerged as one of the pre-eminent public health concerns of the 21st century, in particular as it pertains to pathogenic organisms coupled with the discovery void in area of antibiotics. Medicinal plants have always been a veritable source of bioactive compounds and also serve as 'lead molecules' and templates for the design of medicinally useful synthetic molecules. The present study was designed to investigate the plant *Craterispermum laurinum*, claimed to have antimicrobial activity by folkloric medicine. Ethylacetate and methanol extracts of the dried leaves and flowers were examined phytochemically. Possible antibacterial and antifungal properties were assessed using standard laboratory procedures. Results obtained in this study showed that the extracts of *C. laurinum* contain flavonoids, tannins, saponin, reducing sugars and 2, 6, dideoxysugar. Alkaloids, phlobotannins and anthraquinone components were absent. The antibacterial assay showed prominent activity with zones of inhibition for leaves (50mg/ml) and flower (600mg/ml) extracts against *S. aureus* (18mm, 30mm), *S. faecalis* (26mm, 26mm), *P. aeruginosa* (22mm, 21mm), *E. coli* (26.5mm, 27mm) and *S. typhi* (20mm, 27mm) respectively. The Minimum Inhibitory Concentration (12.8mg/ml, 204.8mg/ml) and the Minimum Bactericidal Concentration (25.6mg/ml, 204.8mg/ml) for leaves and flowers were also determined for the antibacterial assay respectively. Antifungal assay showed activity with zones of inhibition for leaves and flower extracts against *Penicillum species* (21mm, 20mm), *C. albican* (26mm, 24mm) and *S. cerevisiae* (23mm, 22mm) respectively. Sporulation in *A. niger* was inhibited however there was no activity on *A. flavus*. Thus, *C. laurinum* has a promising future as lead agent for the development of potent antimicrobial agent, especially for resistant strains.

***Keywords**: Antibiotics, Resistance, Bioactive, Inhibition, Medicinal*

INTRODUCTION

For thousands of years, medicinal plants have played a significant role in the treatment of a wide range of medical conditions, including infectious diseases. Some naturally occurring chemical compounds serve as models for a large percentage of clinically proven drugs, and many are now being re-assessed as antimicrobial agents. The primary reason for this renaissance is the fact that infectious disease remains a significant cause of morbidity and mortality worldwide, accounting for approximately 50% of all deaths in tropical countries and as much as 20% of deaths in the developed world. Despite the significant progress made in microbiology and the control of microorganisms, sporadic incidents of epidemics due to drug resistant microorganisms and previously unknown disease- causing microbes pose an enormous threat to global public health. These negative health trends call for a global initiative for the development of new strategies for the prevention and treatment of infectious disease, especially, natural products. Literally thousands of plant species have been tested against hundreds of microbial strains in vitro, and many medicinal plants are active against a wide range of gram-positive and gram-negative bacteria, fungi and viruses. However, very few of these medicinal plant extracts have been tested in animal or human studies to determine safety and efficacy (Malady et.al. 2008).

.*Craterispermum laurinum* is used for live fencing as it is fire resistant and easily propagates from cuttings. The plant has many medicinal uses. Common among its purported ethno pharmacological uses are the bark, leaf or root infusion or decoction taken against cough, toothache, fever (including malaria), venereal diseases, high blood pressure and intestinal parasites. Powdered barks, leaves or roots are applied to wounds and sores (Jansen 2005).

METHODS

Plant Material: The plant material i.e. flowers and leaves were harvested from the woods around the University of Lagos, Akoka campus in March 2011. The plant Genus and Family were authenticated by Mr. Aridowo of Forestry Research Institute of Nigeria (FRIN), Ibadan, and the Species was verified through the internet with the aid of the photograph of the plant.

Extraction: The powdered flowers were extracted exhaustively with methanol, using soxhlet apparatus for 48hours. The resulting methanol extract was then concentrated using rotary evaporator until it was a semi-solid mass. These were stored in universal bottles and stored in the fridge pending further use. The methanolic extract was then concentrated with a rotary evaporator at 40^{o}C to about 50mls. The concentrated extract was then defatted by partitioning (rinsing) it with 50mls Hexane thrice (i.e. 3 X 50ml), with the use of a separating funnel. The methanol extract was then dried to solid using hot air oven at 37^{o}C. This was then partitioned with ethylacetate(for the leaves).

The methanolic extract of the flower and ethylacetate extract of the leaves were then subjected to phytochemical screening, Ultraviolet analysis, Chromatographic separation, antibacterial and antifungal potency assay and determination of minimum inhibitory concentrations.

Phytochemical Screening: The plant extracts were subjected to the different phytochemical screening methods including tests for sugars, alkaloids, anthraquinones,phlobotannins, flavonoids, tannins and saponin, using standard methods adapted from Trease and Evans.

Ultraviolet Analysis: The ultraviolet spectroscopy was set to a wavelength range of 200-700nm to scan the wavelength of maximum absorption for the sample. The wavelength of maximum absorption was obtained as 354nm. The instrument was zeroed using the respective solvent of the sample and analyzed individually. Their corresponding wavelength and absorbance were recorded appropriately.

Chromatographic Separation: Different Solvent system was tried and a good solvent mixture which gives a good enough separation of the constituents of the extract was attempted. Various solvent systems were tried and their Rf values were calculated along with their hRf values.

Antibacterial Assay: The plant extracts were subjected to potency assay to determine it potency against selected bacterial consisting of both Gram positive and Gram negative bacteria and they include; *Staphylococcus aureus, Streptococcus faecalis, Pseudomonas aeroginosa, Escherichia coli* and *Salmonella typhi,* using disc diffusion method and intravenous Ciprofloxacin as standard. The minimum inhibitory concentrations for the different bacteria were also determined.

Antifungal Assay: The plant extracts were subjected to potency assay to determine it potency against selected bacterial consisting of both spore forming and non-sporulating fungi and they include: *Penicillum species, Candida albican, Saccharomyces cerevisiae, Aspergillus niger and Aspergillus flavus.*

RESULTS

Phytochemical Screening: Phytochemical Screening of the methanolic extract of the flower *Craterispermum laurinum* indicates the presence of carbohydrates, reducing sugars, keto-sugars, steroidal nucleus, 2, 6, di-deoxysugars, flavonoids,glycosides, saponins, tannins, phlobatannins, free and combined hydoxylhydroquinones. However, the methanolic extract of the flowers of *Craterispermum laurinum* was negative to all alkaloidal tests indicating the absence of alkaloids.
Phytochemical Screening of the ethylacetate extracts of the leaves *Craterispermum laurinum* indicates the presence of carbohydrates, reducing sugars, keto-sugars, steroidal nucleus, 2, 6, di-deoxysugars, flavonoids,glycosides, saponins, tannins, free and combined hydoxylhydroquinones. The ethylacetate extracts of the leaves *Craterispermum laurinum* also test negative to the presence of alkaloids and phlobatannins.

Ultraviolet Analysis: The butanol and ethylacetate extract of the flower of *Craterispermum laurinum* were subjected to ultraviolet spectroscopic analysis. For the butanolic extract,the prominent peaks were observed at 328nm while for the ethylacetate extract, prominent peaks were observed at 310nm. While The butanol and ethylacetate extract of the leaves of *Craterispermum laurinum* were subjected to ultraviolet spectroscopic analysis. For the butanolic extract,the only prominent peaks were observed at 308nm while for the ethylacetate extract, prominent peaks were observed at 312nm and 316nm.

Chromatographic Separation: An attempt was made to isolates the component of the methanolic extract of the flower of *Craterispermum laurinum* using thin layer chromatographic techniques. Thin layer chromatography indicated that some of the constituents of the methanolic extract of flower of *Craterispermum laurinum* can be separated and isolated using appropriate solvent system. Different Solvent system was tried and a good solvent mixture which gives a good enough separation of the constituents of the extract was attempted. Various solvent systems were tried and their Rf values were calculated along with their hRf values. Thin layer chromatography indicated that some of the constituents of the ethylacetate extract of leaves of *Craterispermum laurinum* can be separated and isolated using appropriate solvent system.

Antibacterial Assay: Zones of inhibition were observed with the methanolic extract of the flower of *Craterispermum laurinum* at doses of 600mg/ml, 300mg/ml and 150mg/ml for the different organisms used. While zones of inhibition observed with the ethylacetate extract of the leaves of *Craterispermum laurinum* were observed at 50mg/ml, 25mg/ml and 12.5mg/ml (as indicated in the appendix). The responses obtained were compared to that of standard Ciprofloxacin.
Furthermore, the minimum inhibitory concentration were also determined for each of the organisms using both the methanolic extract of the flowers of *Craterispermum laurinum* and the ethylacetate extract of the leaves of *Craterispermum laurinum.*

Antifungal Assay: Zones of inhibition were observed with the methanolic extract of the flower of *Craterispermum laurinum* at doses of 600mg/ml, 300mg/ml and 150mg/ml for the different organisms used. While inhibition of growth were observed with *Candida albican, Saccharomyces Cerevisiae,* and *Penicillium spp,* spores formation inhibition were observed with *Aspergillus flavus* and *Aspergillus niger*
While zones of inhibition observed with the ethylacetate extract of the leaves of *Craterispermum laurinum* were observed at 50mg/ml, 25mg/ml and 12.5mg/ml (as indicated in the appendix). The response obtained were compared to that of standard Ciprofloxacin.

DISCUSSION

The Phytochemical screening of a plant involves the following: authentication and extraction of plant material; separation and isolation of the constituent of interest through chromatography; characterization of the isolated compounds and quantification evaluation. Parallel to this may be the pharmacological assessment of the separated component.

The methanolic extract of the flower of *Craterispermum laurinum* and the ethylacetate extract of the leaves has been investigated and isolation of the extract so far has not yet afforded pure compounds. However, it has shown prospect of bioactivity.

The preliminary phytochemical screening of the extract of the flower of *Craterispermum larinum* revealed the presence of polyphenolic compounds such as flavonoids, phlobatanin and tannins, saponins, carbohydrates, reducing sugars, keto sugar , steroidal nucleus (ring) and glycosides. It however test negative to alkaloidal test and anthraquinone glycosides, indicating their absence. This is in agreement with its use as dyes, whose major components are tannins. Similarly in line with literature, it is being employed in wounds and sores and as an antimicrobial agent could be attributed to the presence of flavonoids as well as tannins which confers antimicrobial and astringent properties by the sore healing properties respectively.

An attempt was made to isolates the component of the methanolic extract of the flower of *Craterispermum laurinum* using thin layer chromatographic techniques. Thin layer chromatography indicated that some of the constituents of the methanolic extract of flower of *Craterispermum laurinum* can be separated and isolated using appropriate solvent system. Different Solvent system was tried and a good solvent mixture which gives a good enough separation of the constituents of the extract was attempted. Various solvent systems were tried and their Rf values were calculated along with their hRf values.

The butanol and ethylacetate extract of the flower of *Craterispermum laurinum* were subjected to ultraviolet spectroscopic analysis. For the butanolic extract,the prominent peaks were observed at 328nm while for the ethylacetate extract, prominent peaks were observed at 310nm.

The antimicrobial assay of the extract showed zones of inibitions against *Staphylococcus aureus, Streptococcus faecalis, Pseudomonas aeruginosa, Escherichia coli, Salmonella typhi*. The zones observed compared with the standard ciprofloxacin were used to calculate the amount of the extract corresponding to the concentration of the standard. The minimum inhibitory concentrations were also determined for each of the organisms and the minimum bactericidal concentrations were also determined to ascertain that the organisms were actually killed but not just inhibited.

The antifungal assay of the extract also shows some form of inhibition against *Penicillum* species, *Candida albican* and *Saccharomyces cerevisiae*. But has no activity against *Aspergillus niger* and *Aspergillus flavus*. Though inhibited spores formation in *Aspergillus niger*. The zones observed compared with the standard clotrimazole were used to calculate the amount of the extract corresponding to the concentration of the standard.

The ethylacetate extract of the leaves shows potent activity against *Staphylococcus aureus, Streptococcus faecalis, Pseudomonas aeroginosa, Escherichia coli* and *Salmonella typhi* with zones of inhibition. The zones observed compared with the standard ciprofloxacin were used to calculate the amount of the extract corresponding to the concentration of the standard as indicated in the table. The minimum inhibitory concentrations were also determined for each of the organisms and the minimum bactericidal concentrations were also determined to ascertain that the organisms were actually killed but not just inhibited. The extract also showed some activity against some fungi which include *Penicillum species, Candida albican* and *Saccharomyces cerevisiae* with zones of inhibition. While preventing sporulation in *Aspergillus niger* with no activity on *Aspergillus flavus.* The zones observed compared with the standard

clotrimazole were used to calculate the amount of the extract corresponding to the concentration of the standard as indicated in the table.

The phytochemical screening of the plant showed the presence of saponin, tannins, steroidal nucleus, 2, 6-dideoxysugar, carbohydrates, reducing sugar, keto sugar, glycosides, flavonoid,but no alkaloids, Phlobotannins and anthraquinones found.

The UV spectrophotometric analysis showed peaks at 312nm and 316nm. Thin layer chromatography indicated that some of the constituents of the ethylacetate extract of leaves of *Craterispermum laurinum* can be separated and isolated using appropriate solvent system. Various solvent systems were tried and their Rf values were calculated along with their hRf values.

CONCLUSION

From the wok done so far, the biopropect of the plant *Craterispermum laurinum* is not in doubt. The extracts of *Craterispermum laurinum* possess some potent antibacterial and some antifungal activity as observed from the results obtained from the research. The leaves extract showing more activity than the flower extract.

Hence, the result obtained validated the local claim that the plant possesses antibacterial and antifungal activity thus can be used to treat infections of susceptible organisms.

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Zone of inhibition observed with the methanolic extract of the flower of *Craterispermum laurinum*

Organisms	**Zones of inhibition by concentration (mm)**		
	600mg/ml	**300mg/ml**	**150mg/ml**
Escherichia coli	27	23	16
Staphylococcus aureus	30	21	14
Pseudomonas aeruginosa	21	17	14
Streptococcus faecalis	26	19.5	17
Salmonella typhi	27	23	14

The solvent showed no antimicrobial activity

Zone of inhibition observed with standard ciprofloxacin

Organisms	**Zones of inhibition by concentration (mm)**			
	50mg/ml	**25mg/ml**	**12.5mg/ml**	**6.25mg/ml**
Escherichia coli	26	22	19	16
Staphylococcus aureus	40	37	34	29
Pseudomonas aeruginosa	23	21	16	9
Streptococcus faecalis	37	31	28	26
Salmonella typhi	42.5	42.5	42.5	42.5

The solvent showed no antimicrobial activity

Activity of extract with respect to the standard

Organisms	**Concentration equivalent to standard (mg/ml)**		
	600mg/ml	**300mg/ml**	**150mg/ml**
Escherichia coli	51.20	33.50	2.54
Staphylococcus aureus	4.51	-29.57	-56.08
Pseudomonas aeruginosa	30.08	8.82	-7.13
Streptococcus faecalis	5.28	-20.92	-30.99
Salmonella typhi	-	-	-

Equation of the standard curve is y = 4.423x-68.23 (*E. coli*) R^2 = 0.953, y = 3.787x-109.1 (*S. aureus*) R^2= 0.843, y = 5.315x-81.54 (*P. aeruginosa*) R^2= 0.673, y = 4.030x-99.50 (*S. feacalis*) R^2= 0.998

Zone of inhibition observed with the ethylacetate extract of the leaves of *Craterispermum laurinum*

Organisms	**Zones of inhibition by concentration (mm)**		
	50mg/ml	**25mg/ml**	**12.5mg/ml**
Escherichia coli	26.5	23	20
Staphylococcus aureus	18	15	12
Pseudomonas aeruginosa	22	14	9
Streptococcus faecalis	26	22	18
Salmonella typhi	20	18	14

The solvent showed no antimicrobial activity

Zone of inhibition observed with standard ciprofloxacin

Organisms	**Zones of inhibition by concentration (mm)**			
	50mg/ml	**25mg/ml**	**12.5mg/ml**	**6.25mg/ml**
Escherichia coli	26	22	19	16
Staphylococcus aureus	40	37	34	29
Pseudomonas aeruginosa	23	21	16	9
Streptococcus faecalis	37	31	28	26
Salmonella typhi	42.5	42.5	42.5	42.5

The solvent showed no antimicrobial activity

Activity of extract with respect to the standard

Organisms	**Concentration equivalent to standard (mg/ml)**		
	50mg/ml	**25mg/ml**	**12.5mg/ml**
Escherichia coli	48.98	33.50	20.23
Staphylococcus aureus	-40.93	-52.30	-63.66
Pseudomonas aeruginosa	35.39	-7.13	-33.71
Streptococcus faecalis	5.28	-10.84	-26.96
Salmonella typhi	-	-	-

Equation of the standard curve is y = 4.423x-68.23 (*E. coli*) R^2 = 0.953, y = 3.787x-109.1 (*S. aureus*) R^2= 0.843, y = 5.315x-81.54 (*P. aeruginosa*) R^2= 0.673,y = 4.030x-99.50 (*S. feacalis*) R^2= 0.998