

DEFORMATION BEHAVIOUR OF LED AND HILED CURED DENTAL RESIN MICROHYBRID AND NANOFILLED COMPOSITES

Adeleye O.A.^a, Fakinlede O.A.^a, Ajiboye J.S.^b, Adegbulugbe I.C.^c

^aDepartment of Systems Engineering, University of Lagos, Lagos, Nigeria

^bDepartment of Mechanical Engineering, University of Lagos, Lagos, Nigeria

^cDepartment of Restorative Dentistry, College of Medicine of the University of Lagos, Idiaraba, Lagos, Nigeria

ABSTRACT

The deformation behavior of commercial microhybridresin based composite (20/20 composite) and nanofilled composite (Light Cured Universal Composite) cured with the conventional Light Emitting Diode (LED) and exponential Light Emitting Diode (HiLED) under various loading condition have been investigated. Deformation of restorations such as shrinkage and shearing from curing and mastication have been a major concern for clinicians because of void and crack formations in restored tooth structure which affects the mechanical properties of the resin composites. Samples of microhybridresin based composite (20/20 composite) and nanofilled composite (Light Cured Universal Composite) were molded with copper foil molds with standard dimension 2 x 2.5 x 8mm, photo-cured by both conventional Light Emitting Diode (LED) and exponential Light Emitting Diode (HiLED) and then tested on the Electro Force 3200 for their deformation behavior and mechanical properties. Effects of variation of strain rate and curing time were also investigated. The results showed that, out of the four groups of samples studied, microhybridresin based composite (20/20 composite) cured with exponential Light Emitting Diode (HiLED) exhibited highest tensile strength of 28 MPa. The loading and unloading of the samples exhibited hysteresis responses and path dependence nonlinear behavior. At stress values less than 4 MPa, rate dependent recoverable (viscoelasticity) deformation was observed in all the four groups of samples but at stress values beyond 4 MPa rate dependent irrecoverable (viscoplasticity) deformation was observed. Finally, it was observed that increasing curing time leads to increasing tensile strength for materials cured by both methods.

Key words: Deformation, Dental-resin, Microhybrid, Nanofilled, Viscoelasticviscoplastic

1. Introduction

Dental Resin Composites were developed in the late 1950s (Ferreira *et al.*, 2008). They represent a class of materials widely used in restorative dentistry and have found increasing application in modern preventive and conservative dentistry (Jandt and Sigusch, 2009). These materials are also increasingly used for cosmetic purposes such as reconstruction of anterior teeth, correction of stains and erosion, and alignment of teeth. Gold was the first dental restoration material reported in the late fourteen hundreds

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(Sakaguchi *et al.*, 2005, Powers and Sakaguchi, 2006). Many of the materials include noble and base metals and their alloys, amalgam, resin composites, glass ionomers, ceramics, cements, dental waxes, etc. Although chemistry, physics, and other engineering sciences were the fundamental building blocks for developing restorative science, systematic improvement in the mechanical properties were achieved only after principles of mechanics were applied to biological restorative dentistry in the late nineteenth and early twentieth centuries (Powers and Sakaguchi, 2006).

Mechanical testing plays an important role in evaluating fundamental properties of engineering materials as well as in developing new materials and in controlling the quality of materials for use in design and construction. If a material is to be used as part of an engineering structure that will be subjected to a load, it is important to know that the material is strong enough and rigid enough to withstand the loads that it will experience in service. As a result engineers have developed a number of experimental techniques for

mechanical testing of engineering materials subjected to tension, compression, bending or torsion loading. The utilization of the dental resin composites in conservative, preventive or restorative dentistry has progressed significantly over several decades. However, in spite of the superior properties of these materials, polymerization shrinkage and deformation due to mastication are considered to be the major drawbacks of resin composite applications.

The necessary constitutive model for describing the deformation of dental resin composites is not readily available in literature. Hence, there is need to investigate the deformation behaviour in the response of these materials to various loading conditions. Deformation behaviour of a human dentin under *uniaxial compression* including size and rate effects has been studied by He and Swain, (2008), Zaytsev *et al.*, (2011). The study showed that the dentin is mechanically isotropic high elastic and strong hard tissue, which demonstrates considerable plasticity and ability to suppress a crack growth.

In a study carried out by Shah *et al.*, (2009), on Mechanistic aspects of fatigue crack growth behavior in resin based dental restorative composites, the nanofilled composite (Light Cured Universal Composite) showed lower fatigue threshold than the microhybrid resin based composite (20/20 composite). The study only focused on the crack growth behavior and not on the deformation behavior under compression or diametral tensile loading condition. Other studies such as Luana *et al.*, (2013) have investigated the mechanical properties of nanofilled and microhybrid composites cured with conventional Quartz Tungsten Halogen (QTH) for 40s, convention Light Emitting Diodes (LED) for 20s and exponential Light Emitting Diodes (HiLED) for 15s. A lot of research on dental resin composites has shown that the deformation of the material to only curing shrinkage is nonlinear and rate dependent but the deformation of this material due to mastication is still unknown.

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Few of these study include the following: Laughlin, (2003) developed a viscoplastic model for the shrinkage of dental resin composites due to curing and simulated the nonlinear behaviour by using FEM program. Patham, (2009) implemented a viscoelastic model with cure – temperature – time superposition principle into the COMSOL Multiphysics software. The base equations in the software were modified for the implementation. High stresses were also observed in the model results. Dauvillier *et al.*, (2000) and Gambin, (2010) proposed a viscohypoeastic model for the estimation of shrinkage stress in the photo-cured dental restorations. His proposition is based on the Maxwell model, in which the Young's modulus and viscosity were continuous functions of time. An incremental analysis of the process which was carried out enables the formulation of an integral model with an explicit rule for the shrinkage stress for 1D and 3D cases. However, the results gave too high stress values which did not agree with the experimental data and plastic deformation was not considered in his analysis.

Various constitutive models have been developed for rate dependent nonlinear deformation behaviour of polymeric materials. Although a few studies have been done on identifying material deformation behavior and constitutive models for shrinkage of resin based restorative composites, its deformation behavior under uniaxial tensile or repeated loading has not been properly characterized. Hence, the aim of this study is to investigate the mechanical properties and deformation behaviour of the Microhybrid resin based 20/20 composite and Nanofilled Light Cured Universal Composite which are cured with the conventional Light Emitting Diodes for 20 seconds and exponential Light Emitting Diodes for 5 seconds. The effect of curing times on the mechanical properties of the resin composites was also investigated.

The stress relaxation test is a popular way for studying the changes in properties of polymeric material during ageing. Stress relaxation is the behaviour of polymeric materials wherein if a constant strain is applied to this material, the force needed to maintain that strain is not constant, but decreases with time. The process responsible for stress relaxation may be chemical or physical in nature, and under all normal conditions

both types of process will occur simultaneously. However at low or normal temperatures, and/or over a short time, stress relaxation is dominated by physical processes while over long time periods or high temperatures chemical processes are dominant. The key factor in achieving good reproducibility and repeatability while conducting the stress relaxation test is to keep the temperature and compression force constant during all measurements. Polymers are nonlinear and non-Hookean in behaviour because they are viscoelastic. The nonlinearity in polymers can best be described by stress relaxation and a phenomenon known as creep, which describes how polymers strain under constant stress. The cause of Stress Relaxation is that viscous flow in the polymeric material's internal structure occurs by; (a) the polymer chains slowly sliding by each other, (b) by the breaking and reforming of secondary bonds between the chains, and (c) by mechanical untangling and recoiling of the chains.

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2. Materials and Methods

2.1 Materials

Two commercially available particle reinforced resin based dental restorative composites, Filtek Z250 A3 Compules, a Universal Microhybrid Restorative, 20 - .20 Gm. Compules which contains 60 vol% zirconia/ silica fillers with particle size ranging from 0.01 to 3.5_μm (average 0.6_μm) and matrix composition which is made up of Bisphenol A Glycidyl Dimethacrylate (Bis-GMA), Bisphenol A-Ethoxylate Dimethacrylate (Bis-EMA), Urethane Dimethacrylate (UDMA) and Triethyleneglycoldimethacrylate (TEGDMA), and the other restorative composite; Nanofilled Light Cured Universal Fine Hybrid Nano Composite 4g, VITA A1 which contains inorganic fillers; barium glass, silicon dioxide and mixed oxide with the fillers' particle size between 40 nm and 3 μm were used in this study. The total content of fillers is 79 wt% or 61 vol% while its' matrix composition includes Bis-GMA, Urethane Dimethacrylate with 18.8% total monomer content of TEGDMA. It should be noted that both microstructures have a mixture of large and small filler particles, but in the case of the nanofilled the large particles are actually clusters of smaller nanoparticles rather than solid particles.

2.2 Specimen Preparation

The specimens of the two resin based dental restorative composites; microhybrid and nanofilled composites were prepared for testing using an aluminum split molds of dimensions 2 x 2.5 x 8mm as specified by manufacturer's instruction. (Loguercio *et al.*, 2004, Casselli *et al.*, 2006, Juthatip *et al.*, 2006, Luana *et al.*, 2013). The molds were filled with uncured composite and both the top and bottom surfaces were covered with polyester strips that were pressed flat against each side of the mold to remove excess material and provide uniform sample thickness. The aluminum molds filled with uncured composites were then illuminated for polymerization with the light curing units' one after the other at the specified curing times. Two light curing units were used separately for the polymerization of the dental restorative composites, Flash max2, cms, Dental Aps, Copenhagen, Denmark, which emits light with an intensity of through its diodes exponentially (Exponential Light Emitting Diode HiLED) and Flashlite 2.0, denmat which emits light with an intensity of conventionally (Light Emitting Diode LED). The Flash max2 cures for five (5) seconds while the Flashlite 2.0 cures for twenty (20) seconds. The curing was done by placing the tip of the light guide over the center of the mold and the light was activated for curing the specimens. Sixty (60) samples were prepared for all the required tests. The rectangular bar shaped specimens were then taken for the various experiments. The specimens were placed into four groups as specified by different curing modes as Microhybrid cured with Conventional Light Emitting Diodes (LED) continuous exposure to for 20s, Microhybrid cured with Exponential Light Emitting Diodes (HiLED) exposure to for 5s, Nanofilled cured with Conventional Light Emitting Diodes (LED)

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continuous exposure to for 20s, Nanofilled cured with Exponential Light Emitting Diodes (HiLED) exposure to for 5s.

2.3 Testing for Mechanical Properties and Deformation Behaviour

The BOSE® Electro Force (ELF) 3200 testing machine in conjunction with the WinTest® control software located in the Material Testing Laboratory of Systems Engineering Department of University of Lagos was used to conduct the various experiments in uniaxial cyclic loading, uniaxial tension and stepwise loading to determine the mechanical properties and investigate deformation behavior of the dental resin composites. The tensile test on the dental resin composites are carried out in accordance with ASTM standard D7205M – 06(2011) for polymeric materials at (27°C) temperature. The tip-ends of the specimens were glued to the micro-grips in vertical positions on the testing machine. The specimens were subjected to a tensile force (using displacement control of the ELF 3200 Instrument) at a crosshead speed of 0.5 until fracture. maximum tensile strength was obtained from the test. Some samples of Microhybrid dental resin composite were cured with the Conventional Light Emitting Diodes (LED) at different set of times of 20, 30, 40, and 50 secs and were tested for tensile strength in accordance with the ASTM standard aforementioned. As it was done in the earlier test, the tensile tests were carried out at ambient (room) temperature (27°C) in a temperature controlled room. The effect of curing times on the stress-strain behaviour and tensile strength was investigated. The effect of strain rates on the tensile strength of the dental resin composites was also investigated. The tensile tests were done at the following strain rates or crosshead speeds of , , , and until fracture.

Fatigue cyclic tests were done at the same ambient (room) temperature (27°C) using the displacement control tool on the ELF 3200 mechanical testing machine (BOSE® Electro Force (ELF) 3200, 450 KN) between a minimum and maximum load in tension for a prescribed test frequency of 2 Hz. This was chosen because it matches the upper range of the typical human chewing frequency (Shah *et al.*, 2009) and it is also considered clinically relevant. Due to the viscoelastic properties of the resin matrix, higher test frequencies may affect fatigue results. For example, it has been suggested that high test frequencies could lead to internal heating during fatigue testing (Loughran *et al.*, 2005). The fatigue test were done in accordance with ASTM D3479M - 12 for testing for fatigue strength in polymeric materials.

Rate dependent recoverable and irrecoverable deformation of the dental resin composites were determined by the Multiple Stress Creep Recovery (MSCR) tests which were done in accordance with (ASTM D7405 - 08). The Multiple Stress Creep Recovery (MSCR) Tests are temperature dependent, hence they were carried out in a controlled room at ambient temperature (30°C) using the ELF 3200 mechanical testing machine. The Multiple Stress Creep Recovery (MSCR) test involves applying loads in steps and each loading step is followed by recovery period, where no load is applied. Ten loading –

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unloading cycles were applied in the test with the stress level between 5 - 50 MPa. The test applies step loading where one load cycle is comprised of 1.0 second of loading followed by 9.0 seconds of unloading or recovery within each cycle. The MSCR test provides valuable data regarding the rate dependency of the composites. Stress relaxation tests were also done to ASTM standards (ASTM F1276 – 99 (2009)). The stress relaxation tests were carried out at ambient temperature using displacement control of the ELF 3200 Instrument at a crosshead speed of 0.5 to a certain displacement value and then put on hold at that displaced value. At constant displacement (strain), there was progressive decrease in the stress values. This test describes how polymers relieve stress under constant strain.

3. Results and Discussion

3.1 Tensile Strength

The maximum tensile stress before fracture of the four groups of specimens shown in Figure 3.1 (a), (b) are plotted as functions of time and strain respectively. The data were

obtained through the Wintest software installed on the ElectroForce test instrument. Several samples fractured inadvertently prior to successful tests and data collection. It was observed from the results of the tensile tests presented in Figure 3.1 for the four samples of resin composites, that the deformation of the composites to axial loading is geometrically nonlinear and time dependent since it has delayed response to axial loading. The microhybrid cured with the exponential light emitting diode (HiLED) exhibited the maximum tensile stress of , the microhybrid cured with LED exhibited a maximum tensile stress of 27 , the nanofilled cured with HiLED exhibited 23 , while the nanofilled cured with LED exhibited the lowest tensile stress of .

Figure 3.1: (a) Variation of Stress against time for groups of material (b) Stress-Strain behaviour of the tested samples of resin composites

In addition, the test revealed that both materials (microhybrid and nanofilled) cured with exponential light emitting diodes (HiLED) exhibit higher tensile strength than when they

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cured with conventional light emitting diodes (LED). This suggests that the light intensity increases the tensile strength. The result obtained in this study corroborates other works (Luana *et al.*, 2013).

The canonical probe of mechanical properties is the tensile test, whereby a standard specimen is pulled apart in uniaxial tension. The force and displacement are recorded during the test and usually normalized by the specimen geometry to provide a plot of stress versus strain. From this, valuable information about the mechanical behaviour and the engineering performance of the materials can be obtained. Tensile strength test is a common and acceptable test for dental composites (Giannini 2004, Juthatip *et al.*, 2006, Luana *et al.*, 2013). It provides an important indication of the ability of the restorative material to withstand tensile stress generated during mastication. High tensile strength values are important for greater efficiency in supporting occlusal forces.

3.2 Tensile Strength at varying Curing Time

Figures 3.2 (a) and (b) show the developed stresses and tensile strength of the microhybrid cured at different times of 20, 30, 40 and 50 secs. The stresses were plotted as a function of strain while the tensile strength values were taken from tensile strength of failed samples and plotted as a function of time. It was observed that as curing time increases the tensile strength of the cured dental resin increases. It is interesting to see from the results that the tensile strength of the material cured at different times also exhibited nonlinear relation. This tensile strength exhibits asymptotic behaviour for material cured for fifty (50) seconds. There is no increase in the tensile strength as the curing time increases beyond fifty (50) seconds.

(a) (b)

Figure 3.2: (a) Tensile Strength of Microhybrid Cured with LED at different Curing Times (b) Tensile Strength of Microhybrid Compared with Model

Exposure times for polymerization vary depending on the type of light-curing unit and the type, depth, and shade of the composite. Times may vary from 20 to 60 seconds for a restoration of 2 mm thick. Nanofilled composites require longer exposure than

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microhybrid composites because the small filler particles scatter the light more than the larger filler particles. Longer exposure times are needed to obtain adequate depth of cure of nanofilled composites and consequently higher strength. The light intensity at the resin surface is a critical factor in completeness of cure at the surface and within the material. A standard exposure time using most visible lights is 20 seconds (Luana *et al.*, 2013). In general, this is sufficient to cure a light shade of resin to a depth of 2 or 2.5 mm. A 40-second exposure improves the degree of cure at all depths, but it is required to obtain sufficient cure with the darker shades. And as the exposure time increases beyond this point, the ultimate strength becomes asymptotic and at 60- seconds, the increase in ultimate strength becomes insignificant.

3.3 Tensile Strength at varying Strain rates

The results of the tensile test at varying strain rate is presented in Figures 3.3 (a) and (b). The result showed the behaviour of microhybrid cured with LED tested at different strain rates of , , , and . The maximum stresses increased by 6.97, 27.36 and 28.8% respectively as the strain rates increases by a factor of five (5). It is observed from the test that as the strain rate increases, the tensile strength increased. This suggests that the strength of the material is significantly affected by the increase strain rates. It can be observed in Figure 3.3 (b) that as the strain rate increases, there is a corresponding increase in the strains and stresses.

(a) (b)

Figure 3.3: (a)Effect of strain rate on microhybrid cured with LED

(b) Effect of strain rate on stress-strain behaviour

Some materials yield different tensile strengths when tested at different rates of loading and are described as being strain-rate sensitive.

3.4 Fatigue of Microhybrid Cured with LED and HiLED:

The results of fatigue cyclic loading for microhybrid cured with both curing lights shown in Figures 3.4 (a-d) are plots of stresses as functions of strains. These results show that the response of the materials when subjected to repeated uniaxial loading and unloading is path dependent (viscoelasticity). The hysteresis loop shows the amount of energy lost in a loading and unloading circle. As observed in the fatigue cyclic result, hysteresis loop

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is obtained in the stress–strain curve, with the area of the loop being equal to the energy lost during the loading cycle. The hysteresis shows the path dependence of the deformation behavior. This is a characteristic of a viscous material. Since viscosity is the resistance to thermally activated plastic deformation, a viscous material will lose energy through a loading cycle. Plastic deformation results in lost energy, which is uncharacteristic of a purely elastic material's reaction to a loading cycle.

(a) (b)

(d)

Figure 3.4: (a) One cycle of uniaxial loading and unloading of Microhybrid cured with LED

(b) Several cycles of uniaxial loading and unloading of Microhybrid cured with LED

(c) One cycle of uniaxial loading and unloading of Microhybrid cured with HiLED

(d) Several cycles of uniaxial loading and unloading of Microhybrid cured with HiLED

3.5 Rate Dependent Recoverable and Irrecoverable Deformation From Multiple Stress Creep Recovery (MSCR)

The results of the Multiple Stress Creep Recovery (MSCR) tests for the microhybrid cured with HiLED and LED are presented in Figures 3.5(a) and (b). These results showed that, every step load is followed by an unloading or recovery period. A closer observation revealed that, the unloading period returned to zero (0) strains within the first eighteen (18) to twenty (20) seconds of the tests which showed material rate dependent elasticity (viscoelasticity). After this period (i.e. twenty (20) seconds), the strains did not return fully to zero (0) strains which showed that some irrecoverable or permanent deformation had set in. At twentieth (20th) second, the value of strain obtained was 0.02. At this 20th second, the yield point is specified and the yield stress can be obtained from the set of result. As the

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tests progressed, the graphs showed an accumulation and gradual exponential growth in the irrecoverable deformation (strains). This exponential growth suggests a progressive and irrecoverable failure of material after yield point. The exponential growth will continue until the material fractures. The total strain is the sum of the recoverable and irrecoverable strains detected in the deformed material.

(a) (b)

Figure 3.5: MSCR Test Result for Total Strain versus experimental time for MicroHybrid cured with (a) LED and (b) HiLED

The stress values obtained in the Multiple Stress Creep Recovery tests for microhybrid are shown in Figures 3.6 (a) and (b), viscoplastic strains were detected in the tests after eighteen (18) to twenty (20) seconds. Consequently, it can therefore be shown, from Figures 3.6 (a) and (b), that the stress value at the time (eighteen (18) to twenty (20) seconds) the viscoplastic strains were detected was .

(a) (b)

Figure 3.6: MSCR Test Result for Stress versus experimental time for Microhybrid Cured with (a) LED and (b) HiLED

In the case of nanofilled cured with both LED and HiLED, the result as shown in Figures 3.7 (a) and (b), also shows that the unloading period returns to zero (0) strains within the first seven (7) seconds of the tests, after which irrecoverable strains were also detected.

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Similarly, a strain of 0.015 was obtained in the nanofilled material shortly before the irrecoverable strains set in.

(a) (b)

Figure 3.7: MSCR Test Result for Total Strain versus experimental time for Nanofilled cured with (a) LED and (b) HiLED

Similarly, the stress values obtained in the Multiple Stress Creep Recovery tests for nanofilled are presented in Figures 3.8 (a) and (b). From Figures 3.7 (a) and (b), the stress value at the time the viscoplastic strains were detected was MPa. (See Figures 3.8 (a) and (b)).

(a) (b)

Figure 3.8: MSCR Test Result for Stress versus experimental time for Nanofilled Cured with (a) LED and (b) HiLED

The Multiple Stress Creep Recovery (MSCR) tests clearly demonstrated that at stress level lower than 4 MPa, recoverable time dependent (viscoelastic) strains are only present in the material while at higher stress values than 4 MPa, irrecoverable time dependent (viscoplastic) strains begins to accumulate in the material.

3.6 Relaxation Test

The result of the stress relaxation test for the four materials tested is presented in Figure 3.9. From this result, the nanofilled resin composites cured with HiLED lights exhibited

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highest stress relaxation. This was followed by the same material cured with LED. The microhybrid cured with LED exhibited the lowest stress relaxation. This characteristic demonstrates the rigidity and toughness of the cured material.

Figure 3.9: Relaxation Test Results for Microhybrid and Nanofilled cured with both lights

As observed in the MSCR test, the highest viscoplastic strain exhibited by nanofilled cured with LED has also been confirmed by this stress relaxation test. (see Figure 3.9). The Microhybrid cured with HiLED light showed the least relaxation

Conclusions

Based on the investigations of the deformation behavior of microhybrid and nanofilled cured with the Conventional Light Emitting Diode (LED) and Exponential Light Emitting Diode (HiLED) under various loading condition, the following conclusions can be made:

1. These materials exhibit rate dependent nonlinear deformation behavior under various loading conditions. Among the four groups of samples studied, Microhybrid specimens cured with exponential light emitting diode (HiLED) exhibited maximum tensile strength of 28 MPa.
2. The loading and unloading of the samples exhibited hysteresis responses and path dependence nonlinear behavior. When the stress less than , only ratedependent recoverable viscoelastic deformation was present, but as the stress values and loading rates increased beyond 4 MPa, rate-dependent irrecoverable viscoplastic deformation appeared in the deformation.
3. As the strain rates and curing times were increasing, the tensile strength was

increasing. But at curing time beyond fifty (50) seconds, there was no significant increase in tensile strength i.e. tensile strength became asymptotic. In this study, the effect of strain rate is very significant, as seen in the results. It further shows that the rate of loading is important in many materials, particularly polymers and soft tissues.

4. These results showed that the response of cured dental resin composites to various loading conditions is viscoelastic at low repeated forces and viscoelasticviscoplastic at high repeated forces.

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Recommendation

Further experimental study should be conducted to investigate the effects of temperature on the deformation behaviour of dental resin composites.

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DYSLIPIDEMIA PROFILE OF TYPE 2 DIABETIC PATIENTS ATTENDING STATE HOSPITAL IN ILARO, NIGERIA

Adedola Dorcas Wusu*, AbduFattah A. Yusuf, Mutiu Idowu Kazeem

Department of Biochemistry, Lagos State University, Faculty of Science, PMB 0001, Lagos, Nigeria

Abstract

Dyslipidemia is a common feature of diabetic patients, and this condition is a risk factor for the development of cardiovascular diseases. The dyslipidemia patterns were investigated in diabetic patients undergoing medical and dietary treatment. Diabetic patients attending the Diabetes Clinic in State Hospital, Ilaro, Nigeria were recruited into the study after their informed consent. Both anthropometric and biochemical analysis was conducted. Both diabetic normotensive (DBN) and diabetic hypertensive (DBH) subjects had significantly reduced ($p < 0.05$) plasma phospholipids and high density lipoprotein cholesterol (HDL-C), as well as significantly increased ($p < 0.05$) concentration of plasma glucose and low density lipoprotein cholesterol (LDL-C) compared to the non-diabetic subjects. Atherogenic indices (LDL-C/HDL-C and TG/HDL-C) were also significantly elevated ($p < 0.05$) in the diabetic subjects compared to the other groups. This study suggests that dyslipidemia occurred in these diabetic patients due to complications from diabetes mellitus, and this may predispose them to cardiovascular diseases like atherosclerosis.

Keywords: Dyslipidaemia, HDL-cholesterol, phospholipids, hypercholesterolemia, diabetes, LDL-cholesterol.

INTRODUCTION

Dyslipidemia refers to the derangement of one or more of the blood lipoproteins leading to the disequilibrium of lipids concentration within the blood (Misra and Vikram, 2004). Lipids are essential to life, but an excess of certain lipids can increase the risk of

cardiovascular disease (Poirier *et al.*, 2006). In dyslipidemia, the level of one or more of these lipids is abnormal (Poirier *et al.*, 2006). The lipids that are normally measured within the blood include various forms of cholesterol, as well as triacylglycerol. It may manifest as one or more of the following: elevated total cholesterol, low-density lipoprotein cholesterol (LDL-C), and triacylglycerol levels or as decreased high density lipoprotein cholesterol (HDL-C) level with promotion of insulin resistance (Misra *et al.*, 2005).

Dyslipidemia may be as a result of single or multiple genetic mutation that ends up in either overproduction or defective clearance of triglycerides and LDL-C or underproduction or excessive clearance of HDL-C (Rader and Hobbs, 2007). The

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foremost vital secondary reason behind dyslipidemia is inactive life style with excessive dietary intake of saturated fat and cholesterol (Mahley *et al.*, 2008). Other secondary causes include diabetes mellitus, alcohol intake, chronic kidney disease, hypothyroidism, primary biliary cirrhosis and other cholestatic liver diseases as well as drugs such as thiazides, β -blockers, retinoids, highly active antiretroviral agents, estrogen and glucocorticoids (Mahley *et al.*, 2008). Diabetes induced dyslipidemia is not as a result of hyperglycemia intrinsically but primarily as a consequential effect of the defect in lipid metabolism due to insulin resistance. This is so because dyslipidemia is often found in prediabetics and in patients with insulin resistance (e.g., obese patients) but with regular concentration of plasma glucose (Haffner *et al.*, 2000).

Though several studies have been conducted on diabetic subjects, there is a dearth of information on the dyslipidemia pattern of diabetic patients. Therefore, this study was aimed at finding the dyslipidemia pattern of diabetic patients receiving both medical and dietary treatment in Ogun State, Nigeria.

MATERIALS AND METHOD

Chemicals and reagents

The assay kits for the determination of glucose, total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) and triglycerides (TG) were obtained from Randox Laboratories, Antrim BT41, United Kingdom. All other chemicals were of analytical grade and water was glass distilled.

Experimental design

Diabetic patients attending the Diabetes Clinic in State Hospital, Ilaro (Ogun State, Nigeria.) were recruited into the study after their informed consent. A close-ended questionnaire was administered to each patient detailing their lifestyle, eating and drinking pattern as well as family history. Exclusion factors were known patients with any form of diabetic complications and smokers, and patients with family history of hyperlipidemia. Patients who were severely obese or had thyroid, kidney or liver disease and those taking steroids, β -blockers, diuretics, or hypolipidemic drugs were also excluded.

The age range of the subjects was between 20 - 70 years and body mass index (BMI) of the patients were considered. This study was conducted on 111 diabetic patients and 33 non-diabetic controls. The diabetics were stratified into diabetic normotensive (n = 16; 8 males and 8 females) and diabetic hypertensive (n = 95; 32 males and 63 females) and the controls into non diabetic normotensive (n = 19; 10 males and 9 females) and non diabetic hypertensive (n = 14; 8 males and 6 females). Anthropometric parameters like weight, height, body mass index (BMI) and waist circumference were determined.

Systolic and diastolic blood pressures were also taken. About 2% of the diabetics were

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recently diagnosed and were not on any drug while the remaining 95% were on antidiabetic drugs like metformin, glibenclamide and antihypertensive like vasoprin, simvastatin and nifedipine.

Determination of biochemical parameters

Lipids and lipoproteins concentration were determined in plasma samples obtained from blood drawn after 12 h overnight fast. Plasma total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) and triglycerides (TG) were measured by enzymatic methods using commercially available assay kits from Randox Laboratories, UK (Hu *et al.*, 2008). Plasma phospholipid was analysed colorimetrically (Kim *et al.*, 2013) while plasma fasting glucose was assayed using enzymatic glucose oxidase method of Klonoff (2005). Glucose oxidase catalysed the oxidation of glucose to form gluconic acid and hydrogen peroxide. The LDL-C/HDL-C and TG/HDL-C ratios were simply calculated from the respective values of LDL-C, HDL-C and TG.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 5 statistical package (GraphPad Software, San Diego MA, USA). The data were analysed by one way analysis of variance (ANOVA) followed by Tukey test. All the results were expressed as mean $\pm$ SEM and were considered statistically significant when $p < 0.05$.

RESULTS

Table 1 shows the anthropometric parameters of the subjects recruited for this study. The ages, weights and BMI of the female non-diabetic normotensive subjects were significantly reduced ($p < 0.05$) compared to females of other groups. The male diabetic normotensive subjects also differ significantly ($p < 0.05$) in its weight, height and BMI in comparison to other groups. The height of the female diabetic hypertensive patients was significantly reduced ($p < 0.05$) compared to other subjects while the ages of male nondiabetic patients (NDN and NDH) were different from the male diabetic patients (DBN and DBH).

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Table 1: Anthropometric parameters of non-diabetic and diabetic subjects used in this study

Group	Age	Weight	Height	BMI				
	Male	Female	Male	Female	Male	Female	Male	Female
NDN	41.10 ± 8.90 _a	36.00 ± 7.50 _a	62.70 ± 5.10 _a	56.20 ± 8.60 _a	1.62 ± 0.10 _a	1.51 ± 0.10 _a	23.90 ± 3.78 _a	24.54 ± 3.21 _a
NDH	46.00 ± 9.44 _a	49.67 ± 9.30 _b	67.80 ± 7.40 _a	72.70 ± 9.90 _b	1.66 ± 0.10 _a	1.59 ± 0.10 _a	24.82 ± 6.42 _a	28.92 ± 5.89 _b
DBN	27.00 ± 7.10 _b	51.83 ± 8.30 _b	57.00 ± 5.00 _b	66.33 ± 4.70 _b	1.75 ± 0.00 _b	1.55 ± 0.03 _a	18.60 ± 0.00 _b	27.65 ± 1.74 _b
DBH	58.90 ± 7.50 _c	54.87 ± 6.60 _b	71.10 ± 9.00 _a	73.52 ± 8.60 _b	1.66 ± 0.10 _a	1.66 ± 0.08 _b	25.93 ± 3.65 _a	29.50 ± 5.63 _b

Values are mean $\pm$ SEM. Test values down vertical columns carrying different superscript are significantly different ($p < 0.05$).

NDN: Non-diabetic normotensive, NDH: Non-diabetic hypertensive, DBN: Diabetic normotensive, DBH: Diabetic hypertensive, BMI: Body mass index

The systolic and diastolic blood pressures of the subjects is shown in Table 2. Systolic and diastolic blood pressures of all non-diabetic hypertensive and diabetic hypertensive subjects were significantly higher ($p < 0.05$) than that of both non-diabetic normotensive and diabetic normotensive subjects. This trend cut across both the male and female

groups.

Table 2: Systolic and diastolic blood pressures of both non-diabetic and diabetic subjects used in this study

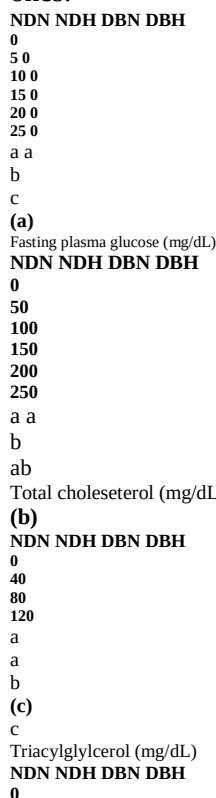
Group	Systolic	Diastolic
Male Female Male Female		
Non-diabetic normotensive	109.30 ± 15.79 ^a	
	102.00 ± 4.47 ^a	71.30 ± 7.38 ^a
	66.70 ± 5.48 ^a	
Non-diabetic hypertensive	130.25 ± 13.48 ^b	
	125.83 ± 9.17 ^b	80.88 ± 6.58 ^b
	83.35 ± 1.65 ^b	
Diabetic normotensive	99.40 ± 5.10 ^a	
	105.67 ± 7.83 ^a	77.00 ± 4.20 ^a
	67.57 ± 4.08 ^a	
Diabetic hypertensive	137.91 ± 20.14 ^b	
	135.48 ± 17.02 ^b	83.34 ± 10.27 ^b
	82.83 ± 9.95 ^b	

Values are mean ± SEM. Test values down vertical columns carrying different superscript are significantly different ($p < 0.05$).

Figure 1 shows the fasting plasma glucose, total cholesterol, triglyceride and phosphoglyceride concentration of diabetic and non-diabetic subjects. There was no significant difference in the glucose concentration of the non-diabetic normotensive and non-diabetic hypertensive groups, but diabetic normotensive and diabetic hypertensive groups witnessed significant increase ($p < 0.05$) compared to the non-diabetic groups (Figure 1a). The total cholesterol concentration of diabetic hypertensive subjects was significantly higher ($p < 0.05$) than other groups, while the results of other groups were

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32 similar to one another (Figure 1b). The diabetic normotensive and diabetic hypertensive subjects respectively exhibited significantly lower and higher triacylglycerol concentration compared to non-diabetic groups (Figure 1c). The phospholipid concentration was similar in both non-diabetic normotensive and non-diabetic hypertensive groups, just as in comparing diabetic normotensive and diabetic hypertensive group to each other (Figure 1d). However, phospholipid concentration was significantly decreased ($p < 0.05$) in the diabetic groups compared to the non-diabetic ones.



200
400
600

a
a
b b

(d)

Phospholipid (mg/dL)

Figure 1: (a) Fasting blood glucose, (b) total cholesterol, (c) triglyceride and (d) phosphoglyceride concentration of non-diabetic and diabetic subjects. Bars with different letters are significantly different ($p < 0.05$).

NDN: Non-diabetic normotensive, NDH: Non-diabetic hypertensive, DBN: Diabetic normotensive, DBH: Diabetic hypertensive

Figure 2 shows the LDL-cholesterol concentration, HDL-cholesterol concentration, as well as LDL-C/HDL-C and TG/HDL-C ratios. The diabetic hypertensive subjects possessed significantly higher ($p < 0.05$) LDL-cholesterol concentration compared to the non-diabetic groups. However, diabetic normotensive subjects were similar to values obtained for other groups (Figure 2a).

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NDN NDH DBN DBH

0

50

100

150

200

a a

ab

(a)

LDL cholesterol (mg/dL)

b

NDN NDH DBN DBH

0

20

40

60

80

100 a

a

b b

HDL cholesterol (mg/dL)

(b)

NDN NDH DBN DBH

0

1

2

3

4

a

a

b

c

LDL-C/HDL-C

(c)

NDN NDH DBN DBH

0.0

0.5

1.0

1.5

2.0

2.5

a

a

a

b

TG/HDL-C

(d)

Figure 2: (a) LDL cholesterol concentration, (b) HDL cholesterol concentration, (c) LDL-C/HDL-C ratio and (d) TG/HDL-C ratio of non-diabetic and diabetic subjects. Bars with different letters are significantly different ($p < 0.05$).

NDN: Non-diabetic normotensive, NDH: Non-diabetic hypertensive, DBN: Diabetic normotensive, DBH: Diabetic hypertensive, LDL-C: Low density lipoprotein cholesterol, HDL-C: High density lipoprotein cholesterol, TG: Triglyceride

The HDL-cholesterol concentration was significantly elevated ($p < 0.05$) in both groups of non-diabetic subjects compared to their diabetic counterparts, but there was no

difference between the non-diabetic normotensive and non-diabetic hypertensive subjects as well as between the diabetic normotensive and diabetic hypertensive groups (Figure 2b). Diabetic hypertensive subjects displayed significantly higher ($p < 0.05$) LDLC/HDL-C ratio, followed by the diabetic normotensive subjects compared to other groups. Both non-diabetic normotensive and non-diabetic diabetic hypertensive group were similar to each other. (Figure 2c). Diabetic hypertensive subjects also possessed significant elevation in TG/HDL-C ratio compared to all other groups, while other groups were similar to one another (Figure 2d).

DISCUSSION

Diabetes mellitus is characterized by derangement in carbohydrate, protein and lipid metabolism which is diagnosed majorly by increase in blood glucose concentration (hyperglycemia) (Kazeem *et al.*, 2015). The observed elevation in the plasma glucose concentration of the diabetic groups, may be due to insulin resistance or insensitivity by the cell membrane receptors in the diabetics compared to the non-diabetic group that has

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normal insulin sensitivity. Glucose is not well metabolized by the cells of the diabetics since it cannot be driven into the cells as result of insulin resistance or insensitivity to glucose by cell membrane receptors (Unger and Orci, 2010).

Previous studies have shown that diabetes is associated with alterations in the plasma lipid and lipoprotein profile, due to hyperglycemia (Maghrani *et al.*, 2004; Krauss, 2004). The difference in total cholesterol concentration between the diabetic hypertensive and other groups may be attributed to the combined effects of diabetes and hypertension. This may be connected to defective lipid metabolism as they cannot properly regulate cholesterol synthesis intracellularly via sterol regulatory element binding protein 2 (SREBP-2) (Pihlajamäki *et al.*, 2004). Therefore in insulin-resistant states such as obesity, metabolic syndrome and type 2 diabetes, cholesterol synthesis is upregulated, and cholesterol absorption efficiency is low.

Triacylglycerol functions as one of the energy reservoirs in man (Voet *et al.*, 2016). The observed increase in the triacylglycerol concentration of diabetic hypertensive subjects compared to other groups is a common feature of type 2 diabetes mellitus. This corroborates the findings of Khan *et al.* (2008). Insulin modulates triglyceride levels, partly by inducing lipoprotein lipase, which contributes to the clearance of chylomicrontriacylglycerol (Kazeem *et al.*, 2015). The rate of chylomicron clearance mostly determines fasting and postprandial triacylglycerol levels in patients with diabetes.

However, many different metabolic abnormalities seem to contribute to diabetic hypertriglyceridaemia, together with increased plasma VLDL concentrations (with or without chylomicronaemia), increased cholesteryl ester transfer protein activity and increased hepatic flux of free fatty acids (Krauss, 2004).

Phospholipid is a class of lipids which are major components of cell membrane and maintain cholesterol homeostasis as well as triacylglycerol secretion and storage (Lagace and Ridgway, 2013). The reduced concentration of total plasma phospholipids observed in the diabetics compared to the non-diabetic subjects may be due to low levels of dihomo- γ -linolenic acid, arachidonic acid, α -linolenic acid and docosahexaenoic acid, experienced in diabetic patients (Hodge *et al.*, 2007). This is because dihomo- γ -linolenic acid and arachidonic acid are precursors of prostaglandin E1 (PGE1) and prostacyclin (PGI2), which are potent platelet anti-aggregators and vasodilators and can prevent thrombosis and atherosclerosis that will lead to hypertension (Das, 2008).

The higher concentration of LDL-cholesterol in the diabetic hypertensive subjects is expected because this group have more oxidized low density lipoprotein that forms foam cells and subsequently, plaque that leads to diabetes-induced hypertension (Nakhjavani *et al.*, 2010). Insulin deficiency or insulin resistance may also be responsible for this because insulin has an inhibitory action on β -hydroxyl- β -methyl glutaryl CoA reductase (HMG-CoA reductase), a key rate-limiting enzyme responsible for the metabolism of

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cholesterol-rich LDL particles (Kumar *et al.*, 2005). This causes increase in fatty acid mobilization from adipose tissues and results in increased production of cholesterol-rich LDL particles. The similarity between the LDL-cholesterol of non-diabetic groups and diabetic normotensive group may be attributed to strict compliance with of the subjects to hypoglycemic control (Murali *et al.*, 2002). This is because oral hypoglycemic agents (e.g metformin) has been shown to have ability to reduce LDL-cholesterol and triacylglycerol concentration (Bolen *et al.*, 2007; Maharani, 2009; Wolffenbuttel *et al.*, 2005).

HDL-C is an anti-atherogenic lipoprotein which transports cholesterol from peripheral tissues into the liver. The HDL-cholesterol concentration of the non-diabetic groups was higher than the diabetic groups, which is in consonance with the findings of Lorenzo *et al.* (2010). This study indicated positive significant association between elevated blood glucose concentrations and low concentrations of HDL-C. Hyperglycemia progressively increases the transfer of cholesterol esters from HDL-C to LDL-C particles (Parikh *et al.*, 2010). In addition, HDL-C is a ready substrate for hepatic lipase that converts it into smaller particles that are readily cleared from the plasma. The relative insulin deficiency that occurs in type 2 diabetes impairs the action of lipoprotein lipase and results in lower HDL-C levels and higher TG levels, which may improve with improved glycemic control (Wang and Peng, 2011). Thus, HDL hypocholesterolemia in type 2 diabetic patients is mainly due to insulin resistance-linked lipoprotein lipase deficiency (Rader and Tall, 2012).

In individuals with diabetes, cardiovascular risk is increased by a cluster of factors such as abdominal obesity, impaired fasting glucose, increased blood pressure, low HDL-C, increase in both TGs and LDL-C particles (International Diabetes Federation, 2009). Although, insulin resistance is crucial to the pathogenesis of this disease, the associated atherogenic lipoprotein phenotype considerably enhances the risk (da Luz *et al.*, 2008). Thus there was the necessity to gauge atherogenic indices from the lipid profile obtained during this study. Many lipoprotein-related indices are postulated to evaluate the chance of cardiovascular diseases in diabetes, which include LDL-C/HDL-C and TG/HDL-C ratios, and are adjudged to be good predictive value for future cardiovascular events (Fisher *et al.*, 2012). The higher values of atherogenic indices (LDL-C/HDL-C and TG/HDL-C) experienced diabetic groups may be due decreased HDL-C and increased LDL-C concentration, which are all connected to derangements in lipid metabolism.

CONCLUSION

The major dyslipidemia observed in diabetic subjects was in reduced phospholipid and HDL-cholesterol, as well as increased LDL-cholesterol concentration. This pattern is a reflection of continuous diabetic control through the administration of antidiabetic drugs. It is therefore recommended that diabetics have their lipid profile checked in addition to glucose level, in order to monitor possible complications that may arise due to dyslipidemia. The non-diabetic hypertensive group also exhibited hypercholesterolemia

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which is undesirable. Non-diabetic individuals should always undergo medical checkup in order to monitor their lipid profile and by extension.

CONFLICT OF INTEREST

The authors declare there is no conflict of interest regarding this study.

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