CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND OF STUDY

Malaria is a disease of global concern; at least 500 million people suffer from this ailment each year (CDC, 1997**).** Malaria is one of the greatest threats to health for international travelers and their contacts. A survey of the cases of malaria in Europe showed an increase from 6840 to 8438 between 1985 and 1995, with case fatality rates as high as 3.6% (Muentener *et al.*, 1999). The situation is worse in sub-Saharan Africa where the problem of malaria has been largely unresolved due to poverty which is linked with unsanitary environmental conditions, a breeding ground for mosquitoes. Poverty also prevents access to the right choice of drugs (White *et al.*, 1999; Phillips, 2001). The problem of malaria, on the health of Africans, has been compounded by HIV/AIDS, a disease characterized by inadequate immune response (Good and Doolan, 1999). By the year 2002, AIDS was stated to be a leading cause of death in Africa (Kates *et al.*, 2002).These are apart from the morbidity and mortality from Tuberculosis which, along with the prevalence of HIV and Malaria infection, create a potential health calamity in the African sub-region (Corbett *et al.*, 2002), and must be remedied.

About 3.1 percent of adults between ages 15 years and 49 years in Nigeria are living with HIV/AIDS (UNAIDS, 2008). With the size of Nigeria's population, estimated around 154 million, it would have meant that by the end of 2007, there were an estimated 2,600,000 people infected with HIV (UNAIDS, 2008).

The estimated figure attributed to deaths from HIV/AIDS in sub – Saharan Africa was 1.6 million in 2007 (UNAIDS, 2007) . With AIDS claiming so many people's lives,

Nigeria's life expectancy has declined. In 1991, the average life expectancy was 53.8 years for women and 52.6 years for men (WHO, 2008). In 2007, these figures had fallen to 46 years for women and 47 years for men.

Despite being the largest oil producer in Africa and the 6th largest in the world, Nigeria is ranked 158 out of 177 on the United Nations Development Programme (UNDP) Human Poverty Index (UNDP, 2008). This poor economic position means that Nigeria is faced with huge challenges in fighting its HIV/AIDS epidemic and malaria.

Interestingly, malaria fever tends to occur when a person's immunity is depressed. Malaria prognosis is also known to be worse in immuno-compromised patients (WHO, 2004a). The combined existence of HIV/AIDS and malaria in Nigeria, given the statistical proportions in which they occur, the accompanying demographic details involved and the socio-economic situation of the country, are major concerns that warrant every effort at combating the two diseases. This has prompted national and international actions against HIV/AIDS and malaria (PEPFAR, 2008; WHO, 2004a). Initial studies suggest that malaria does not occur as an opportunistic infection in HIV infection (Muller and Moser, 1990) and that the incidence of malaria is not more common in HIV infected patients (Dayachy *et al.*, 1991; Muller and Moser, 1990). However, more recent studies have found that malaria is a threat to HIV infected patients. There is an increase in the frequency of malaria cases among HIV/AIDS patients (French *et al.*, 2001; Whitworth *et al.*, 2000). In non-pregnant adults, HIV infection has been found to roughly double the risk of malaria parasitaemia (Whitworth *et al.*, 2000) and clinical malaria. In east and southern Africa, where HIV prevalence is near 30%, it is estimated that about one-quarter to one-third of clinical

malaria in adults can be accounted for by HIV (UNICEF, 2003). Acute malaria infection increased viral load in HIV patients, and one study found that this increased viral load was reversed by effective malaria treatment. This malaria-associated increase in viral load could lead to increased transmission of HIV and more rapid disease progression, with substantial public health implications (UNICEF, 2003).

HIV infection increases the severity of clinical malaria (UNICEF, 2003). Clinical malaria is a febrile condition (Korenromp *et al.*, 2005), HIV/AIDS patients also experience fever. Except there are compelling reasons which indicate otherwise, fever is attributed to malaria in Nigeria. Antimalaria drugs are classified as Over-The-Counter drugs in Nigeria. The implication of this is that individuals are expected to diagnose and treat themselves at least as a first option whenever they perceive an attack of malaria fever. Clinicians tend to carry out presumptive treatment of malaria in patients visiting health facilities in Nigeria. The tendency is for this presumptive therapy to be carried out also on patients with HIV infection whenever they present with fever at the clinic. There is paucity of information on the prevalence of malaria parasitaemia in HIV infected patients that have been diagnosed clinically with malaria fever in Nigeria. The protease inhibitors, saquinavir, ritonavir and indinavir have been proved experimentally to possess antiplasmodia activity. (Skinner- Adams *et al.*, 2004). Knowledge of the antiplasmodial role of other antiretroviral drugs will add to the information of malaria epidemiology among these patients and also serve the potential of new antimalaria drug development. Co-trimoxazole is prescribed as prophylaxis against pneumocystis carinii pneumonia (Hughes *et al.*, 1990; Hudson *et al.*, 1991). Co-trimoxazole has antimalaria property (WHO, 2004a) and has actually been useful as one of the therapeutic options in the management of malaria .The

widespread use of co-trimoxazole among patients with HIV, including its use over a long period of time, is a predisposing factor to the development of resistance by microbes. There is evidence at the molecular level of some degree of *Plasmodium falciparium* cross-resistance between pyrimethamine and trimethoprim (Iyer, 2001). Routine prophylactic use of this antibiotic may therefore lead to resistance formation by malaria parasites to co-trimoxazole and therefore by extension sulphonamides. A sulphonamide of particular interest for which such resistance formation may take place is sulphadoxine and pyrimethamine fixed dose combination (SP). SP is an important drug in the management of malaria because it is one of the common drugs used by patients as self-medication to treat this disease. Also physicians are increasingly prescribing it (Corbett *et al.*, 2002; Iyer *et al.*, 2001; UNICEF, 2003). SP is used as single drug therapy, and as part of a combination therapy with amodiaquine, quinine, or artemisinin. In addition, SP is the drug recommended by the WHO for Intermittent Preventive Therapy of malaria in pregnant women, whether they are HIV positive or negative (WHO, 2004b; UNICEF, 2003).

Further work to verify the level of malaria parasitaemia among patients who are on co- trimoxazole or sulphadoxine will give an indication of the present level of malaria parasite's resistance to these drugs and thus a useful guide in the employment of these drugs while managing patients with HIV.

The effect of the use of other drugs with antimalaria properties, or which have been linked with reduction of malaria parasite density, such as co-trimoxazole, sulphadoxine , and antioxidants superimposed on the effects of the antiretroviral drugs, is not known. Patients in Nigeria have been known to rely on herbs and various

native concoctions, in addition to hospital visits, for treatment of their ailments (Bamidele, 1997; Otubanjo *et al.*, 2000), and may attend multiple clinics where various drugs, unknown to their primary physicians, may be obtained. Since malaria fever is endemic in Nigeria, it is a common reason for self- medication. The care-seeking habit of patients with HIV infection when they feel they have malaria is thus important when assessing self-medication practices. The use of herbs, concoctions, self-medication with orthodox medicines, and visits at multiple clinics such that drugs unknown to the primary physician are taken along with antiretroviral drugs, may lead to drug-drug interactions. Consequently, drug toxicities directed against vital organs particularly the liver and kidneys may occur especially since these patients are already on multiple antiretroviral drugs taken over a long period. This study may provide details of the use of such additional drugs or concoctions and provide knowledge of potential adverse drug interactions and toxicities. Knowledge of the care-seeking practices of these patients in respect of malaria fever will therefore provide information about drug pressure on antimalaria agents and emerging drug resistance.

The use of insecticide treated bed nets (ITNs) has been shown to reduce the incidence of malaria (Takken, 2002). Hence, the use of ITNs has been recommended by the World Health Organization to reduce the incidence of malaria and particularly protect HIV/AIDS patients against malaria. Therefore, donation of ITNs is carried out routinely at the HIV/AIDS clinic in LUTH. There is no study to show the pattern of use of ITNs and other protective measures against malaria among these patients. It will be informative to find out the possible impact of the use of these protective measures on the level of malaria parasitaemia and the clinical presentation of malaria among these patients. It is known that hemoglobin S confers some protection against

malaria among the general population. There is no study to show whether malaria parasitaemia among patients infected with the HIV virus is determined by the immune system or there is a genetic role.

1.2 STATEMENT OF THE PROBLEMS

There is an increase in the frequency of malaria cases among HIV/AIDS patients (French *et al.*, 2001; Whitworth *et al.*, 2000). The importance of this increase is that the HIV/AIDS pandemic is expected to impact the world's malaria situation negatively. Malaria infection could make HIV prognosis worse and increase the infectivity rate (UNICEF, 2003). It is therefore important to know the prevalence of malaria among patients with HIV, for the ultimate purpose of planning appropriate interventions and control programmes against malaria and HIV/AIDS. However, the exact prevalence of malaria parasitaemia among patients with HIV infection in Nigeria is not known. Clinical malaria is a febrile condition (Korenromp *et al.*, 2005).

HIV/AIDS patients also experience fever. The tendency is for clinicians to carry out presumptive treatment of malaria in HIV patients visiting health facilities in Nigeria. This may result in over treatment of malaria with wastage of scarce resources; avoidable adverse drug events and development of antimalaria drug resistance.

Antiretrovirals of the class, protease inhibitors have been demonstrated to possess antimalaria property (Skinner-Adams *et al.*, 2004). The antiplasmodial effects of the antiretroviral drugs commonly used in malaria endemic countries, typified by Nigeria need to be ascertained.

Some drugs, apart from the primary drugs employed in the therapy of malaria, are known to possess antimalaria property (WHO, 2004a).Co-trimoxazole is one drug which has been found useful in patients infected with the Human Immunodeficiency Virus. (Parise *et al.*, 1998; Anglaret *et al.*, 1999). The use of such drugs by these patients will influence the prevalence of malaria parasitaemia among them and may be contributory to the development of resistance by plasmodium to sulphadoxine and pyrimethamine which are still very useful antimalaria drugs.

The Federal Ministry of Health, as part of its contribution to the Roll Back Malaria (RBM) initiative, has been encouraging adequate knowledge and good practices in respect of malaria fever (FMOH, 2000). Prophylactic measures, such as the use of ITNs, indoor mosquito spraying, rational use of antimalaria drugs by patients and visits to the appropriate health facility for consultations when patients have malaria fever are some of the key points in controlling malaria through the RBM programme. A previous study in Enugu has shown that official malaria treatment policies may have little impact at consumer level (Harrison *et al.*,2004). The amount of knowledge and level of compliance to these practices or otherwise by patients with HIV in respect of malaria will impact the entire RBM programme significantly, since these patients constitute a significant subgroup of malaria prone population (Whitworth *et al.*, 2000).

1.3 AIM AND OBJECTIVES

The aim of the study is to investigate interactions between malaria and HIV/AIDS, the role of preventive measures and drug use by HIV patients on parasitaemia.

The specific objectives of this study include:

1. To investigate the knowledge and care-seeking practices of malaria in HIV infected patients.
2. To determine the pattern and effect of use of preventive measures against malaria among these patients.
3. To determine the prevalence of malaria parasitaemia among HIV infected patients.
4. To determine the proportion of clinically diagnosed malaria cases with laboratory confirmation of malaria parasitaemia.
5. To determine the influence of co – trimoxazole, sulphadoxine/pyrimethamine, artesunate, and artemisinin based combination therapy on the malaria status of HIV infected patients.
6. To establish whether there is any relationship between HIV and malaria.

1.4 SIGNIFICANCE OF STUDY

By this study, the malaria status of patients with the Human Immunodeficiency Viral infection placed on different antiretroviral drugs which are usually prescribed at the HIV/AIDS clinic, LUTH will be investigated. It is expected that the influence of different preventive measures, such as insecticide treated bed nets, use of drugs with antimalaria property on the malaria status of these patients, will also be illustrated. The link between malaria parasitaemia and some haematological parameters will be investigated. This study will provide a better understanding which may be useful for control of malaria among patients with HIV infection in a malaria endemic region.

1.5 LIMITATION OF STUDY

The major limitation of this study is that it investigated patients with Human Immunodeficiency Virus infection. There is a lot of stigmatization of these patients and their families in the society; hence they were rather reluctant to be recruited for research. It was necessary to exercise extra caution during this work as a protective measure for the investigator.

1.6 DEFINITION OF TERMS

- **AIDS –** Acquired Immune Deficiency Syndrome
- **Artemisinin-based combination therapy**- the use of artemisinin or its derivative administered with another antimalaria drug for the purpose of curing a malaria attack.

- **Cross resistance between pyrimethamine and trimethoprim** - the development of resistance by microrganisms to pyrimethamine because of an initial resistance to trimethoprim or vice versa.

- **Hematological parameters** - markers of hematological functions which include hemoglobin concentration, CD_4 count, also genotype and blood group.
- **HIV -** Human Immunodeficiency Virus
- **Intermittent Preventive Therapy** – the use of the antimalaria drug sulphadoxine/pyrimethamine by pregnant women twice or thrice for preventive purposes.

- **Over-the-Counter drugs**- means drugs which a person can buy without a doctor's prescription.
- **Presumptive treatment of malaria** - the use of antimalaria agent after a diagnosis has been made based on patients' clinical presentation in the absence of blood smear for malaria parasite.
- **Sulphadoxine and primethamine fixed dose combination** – two antimalaria agents compounded together as one in a fixed ration.

1.7 LIST OF ABBREVIATIONS

AIDS – Acquired Immune Deficency Syndrome

CDC – Center for Disease Control

ECG – Electrocardiography

FMOH – Federal Ministry of Health

HIV – Human Immunodeficiency Virus

IFN - Interferon

IL – Interlukin

ITN – Insecticide Treated Net

LUTH – Lagos University Teaching Hospital

PEPFER – United States President's Emmergency Plan for AIDS Relief

RBM – Roll Back Malaria

VSA – Variant Surface Antigen

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The Link Between HIV/AIDS and Malaria

2.1.1 Demographic Considerations

Malaria and AIDS are two important global health problems of our time. Together, they cause more than four million deaths per year. These two diseases accounted for over 3 million deaths in 2007 (WH0, 2008; UNAIDS, 2007) Malaria accounts for more than a million deaths each year (Hochman and Kim, 2009; WHO, 2008), of which about 90% occur in tropical Africa, where malaria is the leading cause of mortality in children (Murphy and Breman, 2001; Samba, 2001), especially those below five years. In Nigeria, malaria accounts for 30 - 50% morbidity and 25% mortality in under fives (FMOH, 2000). Apart from young children, pregnant women are among the most affected by the disease (ter Kuile *et al.*, 2004; WHO, 2004b).

Constituting 10% of the overall disease burden, malaria places a substantial strain on health services and Africa looses about USD 12 billion of production cost each year. Sub-Saharan Africa is also home to more than 29 million people living with HIV/AIDS. The estimated overall prevalence is 5% in sub-Saharan Africa (Hochman and Kim, 2009), with some countries reporting prevalence rates of greater than 25%. In 2003 in Africa, AIDS claimed the lives of an estimated 2.4 million people and over 600, 000 children were newly infected with the virus. While new HIV infections in adults and children have decreased since 2005, there were an estimated 2.5 million children living with HIV in 2007, nearly 90% of whom are in sub-Saharan Africa. It is estimated that 2.1 million deaths in 2007 were due to HIV/AIDS, of which 1.6

million occurred in sub-Saharan Africa, making HIV/AIDS the number one cause of mortality in the region (UNAIDS, 2007). Malaria in HIV infected children under five years of age (Mermin, *et al*., 2004) in heavily affected countries increasingly accounts for a large proportion of mortality. By taking its greatest toll on its young and most productive generation, HIV and AIDS hinder sustainable development in Africa. Malaria and HIV/AIDS are both diseases of poverty and causes of poverty and they share determinants of vulnerability (Good and Doolan, 1999; ter Kuile *et al*., 2004; Mwapasa *et al*., 2004). Malaria and HIV/AIDS are highly endemic and there is wide geographical overlap in sub-Saharan Africa (French and Gilks, 2000; Chandramohan and Greenwood, 1998).

Among the most affected countries are Cameroon, Central African Republic, Malawi, Mozambique and Zambia, where more than 90% of the population is exposed to malaria and HIV prevalence among adults 15-49 years is above 10%. Outside Africa, the two diseases overlap in certain at-risk groups in South-East Asia and Southern America (Bastos *et al*., 1999; Chau *et al*, 2002) and in several Indian cities such as Mumbai (Khasnis and Kamad, 2003). Given the wide geographic overlap in occurrence and the resulting co-infection, the interaction, between the two diseases clearly has major public health implications (Hewitt *et al*., 2006). Also, the consequences of the interactions between Malaria and HIV/AIDS are particularly serious for reproductive health. In areas with stable transmission in sub-Saharan Africa, approximately 25 million pregnant women are exposed each year to malaria (Menendez *et al*., 2007). About 10.5 million of these become infected with malaria in the second or third trimester (ter Kuile *et al*., 2004). HIV-infected pregnant women are more likely to develop clinical malaria. It is estimated that if the average HIV prevalence among pregnant women in sub-Saharan Africa is about 9%, an additional

500, 000 women will have malaria infection during pregnancy. As HIV prevalence increases, the number of malaria cases during pregnancy attributable to HIV increases as well.

It is unclear whether malaria during pregnancy increases the risk of mother-to-child transmission of HIV, as studies examining this relationship have shown conflicting results. In sub-Saharan Africa, human immunodeficiency virus (HIV) and malaria are among the leading causes of morbidity during pregnancy (ter Kuile *et al.*, 2004). A review of studies shows that HIV impairs the ability of pregnant women to control malaria parasitaemia. Results from 11 studies showed that HIV infected women experienced consistently more peripheral and placental malaria (summary relative risk = 1.58 and 1.66, respectively), higher parasite densities, and more febrile illnesses, severe anaemia, and adverse birth outcomes than HIV uninfected women, particularly in multigravidity. Thus, HIV alters the typical gravidity-specific pattern of malaria risk by shifting the burden from primarily primigravidity and secundigravidity to all pregnant women. The proportional increase of malaria during pregnancy attributable to HIV was estimated to be 5.5% and 18.8% for populations with HIV prevalence of 10% and 40%, respectively. Since co-infected pregnant women are at very high risk of anaemia and malarial infection of the placenta, a considerable proportion of children born to women with HIV and malaria infection have low birth weight and are more likely to die during infancy (Bloland *et al.*, 1995; Van Geertruyden *et al.*, 2004). Maternal malaria was associated with a two-fold higher HIV-1 viral concentrations (ter Kuile *et al.*, 2004). It is unclear whether malaria during pregnancy increases the risk of mother-to-child transmission of HIV, as studies examining this relationship have shown conflicting results. Three studies investigating whether placental malaria increased mother-to-child HIV-1 transmission showed conflicting results, possibly

reflecting a complex balance between placental malarial immune responses and stimulation of HIV-1 viral replication. Further investigations of interactions between antiretroviral drugs, prophylaxis with co-trimoxazole, and antimalarial drugs in pregnant women are urgently needed. There is a clear need to strengthen the deployment of existing malaria and HIV prevention and intervention measures for pregnant women. HIV/AIDS may augment the risk of malaria illness, especially in those with advanced immunosuppression (French *et al.*, 2001).

In areas of unstable malaria transmission, HIV-infected adults may be at increased risk of developing severe malaria. HIV infected adults with low CD_4 cell counts may also be more susceptible to treatment failure of antimalarial drugs. Furthermore, acute malaria episodes temporarily increase viral replication and hence HIV viral load. As an important cause of anaemia, malaria frequently leads to blood transfusions, which is a potential risk factor for HIV infection (WHO, 2004a).

In addition, it is known that with time, people living in malaria endemic regions acquire immunity against malaria to the extent that they may not succumb to malaria attacks as easily as other people who are newly exposed; just as the S haemoglobin in individuals with the AS genotype confers some immunity against malaria. These immunity factors may also modulate the incidence and outcome of malaria among patients with HIV/AIDS (Tracy and Webster, 2001).

2.1.2. THE ECONOMIC BURDEN OF HIV/AIDS, MALARIA AND TUBERCULOSIS

Ill-health contributes to impoverishment, a process brought into sharper focus by the impact of the human immunodeficiency virus / acquired immunodeficiency syndrome (HIV/AIDS) epidemic. Studies have measured the economic costs and consequences of illness for households, focusing on malaria, tuberculosis (TB), and HIV/AIDS (Russell, 2003). It is clear that in resource-poor settings, illness imposed high and regressive cost burdens on patients and their families. Direct and indirect costs of illness for malaria has been found to be less than 10% of the household income, but still significant when combined with the costs of other illnesses. The costs of TB and HIV/AIDS were catastrophic for households (more than 10% of the income). Health service weaknesses in many countries, including low coverage, user charges, and poor quality of care, contributed to high costs. Poor households in developing countries with a member with TB or HIV/AIDS struggled to cope, highlighting the urgent need for a substantial increase in health sector investment to expand access to preventive and curative health services. Government and non-governmental interventions should also be broadened to encompass measures that reduce the substantial indirect costs associated with diseases such as malaria, TB, and HIV/AIDS.

2.1.3 PUBLIC HEALTH IMPLICATIONS OF CO-INFECTION WITH MALARIA AND HIV/AIDS

The association between both infections has important implications. Malaria and HIV-1 are 2 of the most common infections in sub-Saharan Africa and, to a lesser extent, in other developing countries. It is estimated that 38 million Africans are infected with HIV-1 (UNAIDS, 2004), whereas 300 million to 500 million suffer

from malaria each year (WHO, 2006). Therefore, any interaction between these infections will have a significant public health effect, even if the statistical effect is modest. On a population basis, an increased prevalence of malaria and increased parasite density in HIV-infected individuals could lead to increased malaria transmission affecting both HIV-positive and negative individuals (Whitworth *et al.*, 2000). This assumes that the frequency, duration, and density of gametocytemia rise in parallel with asexual parasitaemia, which is currently unproven. The increased risk of clinical malaria in HIV-positive subjects could increase the burden on clinical services in areas where HIV-1 is prevalent.

The population-attributable fraction of adult malaria due to HIV-1 would be expected to rise in parallel with HIV-1 prevalence. In a region with an HIV-1 prevalence of 30%, such as parts of southern Africa, the population-attributable fraction could reach 20% for parasitaemia and 35% for clinical malaria. However, malaria tends to affect mainly children and pregnant women, especially in rural areas, whereas HIV is more common among sexually active adults in urban centers. This mismatch, coupled with the fact that malaria tends to be more intense in western and eastern Africa whereas HIV predominates in southern Africa, means that the high population-attributable fractions given above will not prevail across the entire continent. Indeed, a computer-simulation-modeling study estimated that HIV would increase the incidence of clinical malaria and malaria deaths across the continent by <5 %. (Korenromp *et al.*, 2005).

Nevertheless, in regions of unstable malaria in southern Africa, the HIV-attributable increase might reach 28% for clinical malaria and 114% for malaria deaths.

2.1.4. IMPLICATIONS FOR CLINICAL AND PUBLIC HEALTH MANAGEMENT

In endemic areas, the most relevant immediate action is to encourage HIV-infected patients to avoid malaria because of their increased risk of infection and clinical disease. Clinicians should advise their HIV-infected patients to avoid mosquito bites, perhaps best achieved by sleeping under an insecticide-impregnated bed net (Whitworth, 2006). Alternatives include using mosquito repellents on skin or clothing or sleeping in a room with burning mosquito-repellent coils or tablets. These alternatives are likely to be too expensive for regular use by people living in endemic areas, but may be considered by visiting travelers. Of course, visitors to malaria endemic zones should take prophylaxis, whether they are HIV infected or not.

The use of antimalarial chemoprophylaxis should be stressed in endemic areas (WHO, 2004a). People living with HIV in such areas may be understandably reluctant to take regular preventive medications, but at-risk groups such as pregnant women and their fetuses are particularly likely to benefit. Intermittent presumptive treatment with at least 3 doses of sulfadoxine-pyrimethamine (SP), given monthly at routine prenatal clinic visits during the second and third trimesters of pregnancy, is the most practical public health approach for preventing malaria-related maternal anaemia, low birth weight, and the subsequently higher risk of infant mortality. (Parise *et al.*, 1999) Clinicians must be aware that HIV infection increases the risk of reinfection with malaria within 28 days of starting antimalaria treatment. Pharmacovigilance and additional evidence about the efficacy and safety of antimalaria drugs in HIV infection are urgently needed.

As a result of studies of co-trimoxazole (trimethoprim-sulfamethoxazole) prophylaxis showing significant reductions in morbidity and mortality among HIV-infected adults, (Anglaret *et al.*, 1999; Wiktor *et al.*, 1999) daily co-trimoxazole prophylaxis is recommended for all symptomatic adults and children living with HIV in Africa. The antifolate drug combination co-trimoxazole is similar to SP and has a similar effect on malaria parasites. There is a risk that widespread use of co-trimoxazole will hasten the development of resistance to SP in malaria parasites, given some evidence of *P falciparum* cross-resistance between trimethoprim and pyrimethamine at the molecular level (Iyer, 2001). This may potentially accelerate resistance to SP, which is still being used for the treatment of malaria in Africa as single drug therapy. Sulphadoxine is used as part of Artemisinin-based combination therapy and in antenatal intermittent preventive therapy (IPT) programmes. Conversely, expanding the use of SP in Africa might further the development of resistance to co-trimoxazole by other pathogens, such as Streptococcus pneumonia. The effectiveness of both of these drugs needs to be carefully monitored in country programmes.

The World Health Organization recommends that pregnant HIV-infected women should not receive intermittent presumptive treatment with SP if they are already receiving co-trimoxazole prophylaxis (WHO, 2006). It also follows that HIV-infected individuals receiving co-trimoxazole prophylaxis should be treated with antimalaria drugs other than SP. Some evidence indicates that provision of co-trimoxazole prophylaxis to HIV-infected persons substantially reduces transmission of malaria to other household members. (Mermin *et al.*, 2005)

A recent study in Uganda showed that these antimalaria measures can be successfully implemented for HIV-infected adults in field conditions (Mermin *et al.*, 2006). The

combination of regular co-trimoxazole prophylaxis, provision of Antiretroviral therapy, and use of insecticide-impregnated bed nets were associated with a 95% reduction in the incidence of febrile episodes of malaria.

2.1.5. PHYSIOLOGIC IMPACT OF HIV ON THE IMMUNE SYSTEM

HIV infects and depletes CD_4+ T lymphocytes, putting patients at risk for opportunistic infection and malignancy, the major causes of death due to HIV and AIDS. However, it also has effects on the systemic inflammatory response, causing activation and/or apoptosis in a variety of immune cells as well as elevated levels of pro-inflammatory cytokines and chemokines in plasma and lymph nodes. This immune activation, rather than being a reflection of antiviral immunity, is associated with HIV-1 disease progression (Appav and Sauce, 2008). It is also a potential means by which HIV affects disease course and outcome in other infections, such as malaria. Pro-inflammatory cytokines play an important role both in control and pathogenesis of HIV infection. During infection, viral particles are taken up by antigen presenting cells (APCs), which are then recognized by CD_4+ T lymphocytes, causing activation and release of interlukin-2 (IL-2) and interferon-gamma (IFN-gamma).

These pro-inflammatory cytokines in turn stimulate CD_8+ T lymphocytes which control viremia. It is not known if this inflammatory response seen in HIV has an effect on the adherence and sequestration seen in malaria. However, HIV upregulates adhesion molecules on endothelial cells, which may compound the adherence and sequestration seen in malaria (Gendelman *et al.,* 2005). HIV also dysregulates pathways of cytokine expression, such that the production of the pro-inflammatory cytokines IL-12 and IFN-gamma is decreased, and expression of the anti-inflammatory cytokines IL-10 is increased (Yaday *et al.*, 2009). As HIV progresses

clinically to AIDS, there are effects on innate immunity, with progressive loss of T lymphocyte responses to common recall antigens (Clerici and Shearer, 1993). Increased IL-10 has been shown to play a role in this impaired innate immune response in AIDS patients (Brown *et al.*, 2000). The impaired innate immune response in patients with AIDS may in part account for the increased rates of symptomatic malaria seen in cohort studies. However, the decreased production of IL-12 and IFN-gamma seen in HIV is confounding, as high levels of these pro-inflammatory cytokines are associated with severe malaria in clinical studies (Prakash *et al.*, 2006) and previously mentioned cohort studies have found higher rates of severe or symptomatic malaria in subjects with HIV. Increased expression of IL-10 also appears to play a role in loss of adaptive immunity. IL-10 impairs T helper type 1 (Th1) responses (Brown *et al.*, 2000). Dendritic cells in HIV/AIDS are functionally impaired, producing less IL-12 and more IL-10, disrupting the IL-12/IFN-gamma signaling pathway and contributing to problems with adaptive immunity (Yaday *et al.*, 2009). It is not clear if this impairment is due to direct HIV infection of dendritic cells, or that indirect effects of chronic antigenic stimulation or exposure to virally induced proteins cause dendritic cell dysfunction. Given the important role of dendritic cells in adaptive immunity to malaria, the effects of HIV on dendritic cell dysfunction may also contribute to the higher frequency of symptomatic parasitaemia seen in cohort studies.

In a cohort study in Kenya, HIV co-infected women had higher placental parasite densities and higher rates of antenatal malaria transmission than did women without HIV infection (Perrault *et al.*, 2009). Maternal antibody to variant surface antigens (VSAs) on malaria-infected erythrocytes plays an important role in pregnancy-related immunity to malaria. Sera from HIV-infected mothers, when analyzed by flow

cytometry contained fewer antibodies to VSAs in both placental and pediatric isolates of malaria than did sera from HIV-uninfected mothers (Dembo *et al.*, 2008). Additionally, assays using plasma or purified IgG from HIV-infected or HIV uninfected primi- or multigravidae women found that HIV-uninfected multigravidae women had high levels of opsonic phagocytosis of infected erythrocytes which was due to IgG1 and IgG3 specific for VSA. Opsonic phagocytosis was not seen with plasma or purified IgG from HIV-uninfected primigravidae women or HIV-uninfected men. HIV-infected multigravidae women had significantly lower plasma opsonizing activity than did their HIV-uninfected counterparts (Keen *et al.*, 2007). Cohort studies in Cameroon show that malaria infection during pregnancy may increase the risk of mother-to-child transmission of HIV(Avouba *et al.*, 2003; Avisi *et al.*, 2004). One potential mechanism for this was evaluated in vitro, where binding of recombinant *P. falciparum* adhesion to chondroitin sulfate A on human placental cells increased HIV-1 replication in those cells, possibly via TNF-alpha stimulation (Avouba *et al.*, 2008).

2.1.6. DRUG RELATED INTERACTION BETWEEN HIV AND MALARIA

Aspartic proteases play key roles in HIV and are thus important therapeutic drug targets. Aspartic proteases are also important in malaria parasites (as plasmepsins), and antiretroviral protease inhibitors seem to have both direct effects on Plasmodium and indirect effects related to cytoadherence and phagocytosis. The antiretroviral protease inhibitors saquinavir, ritonavir, indinavir, nelfinavir, amprenavir, lopinavir, and atazanavir directly inhibit erythrocytic stages of *P. falciparum* grown *in vitro* at concentrations achieved *in vivo* (Skinner-Adams *et al.*, 2004; Parikh *et al.*, 2005), and mice infected with *P. chabaudi* had a delay in potency and attenuation of parasitaemia

when given oral ritonanvir/saquinavir or ritonavir/lopinavir (Andrews *et al.*, 2006). Some antiretroviral drugs also seem to exert an effect on the pre-erythrocytic stages of malaria. Using *P. berghei*, a rodent strain of malaria, saquinavir and lopinavir inhibited development of extra-erythrocytic liver stages *in vitro*. *In vivo* mouse studies, using the rodent strain *P. yoelii* showed a reduction in liver parasite burden when lopinavir/ritonavir was administered (Hobbs *et al.*, 2009). The antiretroviral protease inhibitors ritonavir and saquinavir affect CD_{36}-mediated cytoadherence, thought to play a role in cerebral malaria and other end-organ damage in severe malaria. The drugs decrease CD_{36} surface concentrations on C32 epithelial cells in culture which was associated with a decrease in cytoadherence of parasitized erythrocytes (Nathoo *et al.*, 2003). No effect on ICAM-1 expression on cells was seen. In addition, these protease inhibitors affect nonopsonic phagocytosis of parasitized erythrocytes. Human macrophages exposed to ritonavir or saquinavir also had reduced surface values of CD_{36}, with an associated decrease in non-opsonic phagocytosis. The non-nucleoside reverse transcriptase inhibitor nevirapine had no effect on CD_{36} concentrations on either C32 cells or macrophages and did not affect cytoadherence or phagocytosis. The antimalarial drug, chloroquine, has effects on HIV, inhibiting the production of infectious viral particles by impairing virus glycosylation. Chloroquine also has synergistic effects on HIV suppression with the protease inhibitors indinavir, ritonavir, and saquinavir at concentrations achieved with prophylaxis dosing (Savarino *et al.*, 2004). *In vitro*, studies have also shown a synergistic effect on malaria growth between the protease inhibitors ritonavir and saquinavir and both chloroquine and mefloquine (Skinner-Adams *et al.*, 2007).

An additional drug-related interaction between malaria and HIV involves the use of trimethoprim-sulfamethoxazole prophylaxis in HIV-infected patients. In rural

Uganda, HIV-infected participants given trimethoprim-sulfamethoxazole had a 76% decrease in rates of malaria compared with when they were not receiving trimethoprim-sulfamethoxazole, and participants who received antiretroviral therapy plus trimethoprim-sulfamethoxazole had a 92% decrease in malaria rates (Mermin *et al.*, 2006). It is important to note that the antiretroviral regimen used did not include a protease inhibitor.

2.1.7. COMPARISM OF HIV IMPACT ON MALARIA BETWEEN SOUTHERN AFRICA AND OTHER AFRICAN COUNTRIES

Across 41 countries in sub-Saharan Africa, the HIV-1 epidemic may have increased the incidence of clinical malaria by 1.3% (95% CI 0.6%–7.9%) and malaria deaths by 4.9% (95% CI 3.1%–17.1%) in 2004. Continentwide impact was limited by the different geographic distributions of the 2 diseases and their different age patterns.

For southern African countries namely South Africa, Zimbabwe, Namibia, Swaziland, estimated proportional increases were $\leq$28% (95% CI 14%–47%) for malaria incidence and $\leq$114% (95% CI 37%–188%) for malaria deaths. An impact of HIV-1 of this magnitude may have contributed to observed increases of malaria in the 1990s in areas of unstable transmission, including Kwa-Zulu Natal (Craig *et al.*, 2004, Tsoka *et al.*, 2002) and northern Zambia (Sharp *et al.*, 2002). Outside southern Africa, however, HIV-1 is unlikely to be a major contributor to rises in malaria, and where this appears to be so, a more plausible explanation may be over diagnosis of fevers as malaria in HIV-1 patients. Such over-diagnosis may occur unintentionally in settings where malaria is diagnosed without parasitologic confirmation because of the increased frequency of acute fevers in HIV-1 patients (French *et al.*, 2001).

Intentional misdiagnosis could also occur if doctors are reluctant to diagnose illness as HIV-related for fear of social stigma. These estimates have several limitations. First, the magnitude of effects of HIV-1 on malaria incidence and death risk in individual patients is critical, but uncertain because of diagnostic problems in settings of high malaria transmission and a lack of population-based data from areas of low intensity and unstable transmission (Korenromp *et al.*, 2005).

Second, results are sensitive to age patterns in malaria, which are not well known. The sharp contrast in estimated impact of HIV-1 between the 5 southern African countries and the remainder of Africa depends on the assumption that malaria declines more slowly with age in South Africa, where all malaria is assumed to be unstable. In practice, the shift from unstable to stable malaria transmission, from clinical effects in all age groups to a predominance in young children, is more gradual; thus, effects of HIV on malaria in Zimbabwe and Zambia, for example, may be more similar than we estimated. The estimation method developed here could, nevertheless, be applied to more refined age-specific estimates of malaria incidence and death (Korenromp *et al.*, 2005).

Finally, sub national heterogeneity in malaria or HIV, apart from urban/rural differences, was not considered, and this fact may have biased the estimation for countries where either or both diseases are heterogeneously distributed, such as Kenya, Ethiopia, Tanzania, and South Africa (Craig *et al.*, 1999). For example, in South Africa, both malaria and HIV-1 are concentrated in Kwa-Zulu Natal, so that their interaction may be greater than our estimate.

The impact of HIV-1 estimated only pertains to malaria cases and deaths and does not include effects on anemia or adverse birth outcomes attributable to concurrent malaria

and HIV-1 in pregnant women. In areas of high-intensity transmission such as in Kenya and Malawi, the latter effects might be more important than malaria cases and deaths per se. Also, this analysis did not cover the effect of HIV-1 on demand for antimalarial drugs. In most of rural Africa, antimalaria drugs are presumptively prescribed to treat any fever without an obvious non-malarial cause. Recurrent fevers in HIV-1 patients may, therefore, cause considerable overuse of antimalarial drugs, increasing not only costs but also the risk for drug resistance. The HIV-1 epidemic thus underlines the need to improve capacity for laboratory diagnosis of febrile disease in Africa.

To limit the impact of HIV-1 on malaria, HIV-infected persons, in addition to young children and pregnant women, may form a target group for provision of insecticide-treated mosquito nets (WHO, 2004a). In areas of low intensity and unstable transmission, HIV may be a reason for intensifying or resuming indoor residual spraying to control malaria vectors. For HIV-infected persons who are prone to treatment failure with conventional antimalarial drugs (Colebunders *et al.*, 1990; Birku et al., 2002; Kamya and Kigonyal, 2001; Muller and Moser, 1990), effective combination therapy is of utmost importance.

Highly active antiretroviral combination therapy has great potential to reduce HIV-related malaria (Seyler *et al.*, 2003). Co-trimoxazole prophylaxis, recommended for adults and children living with HIV in Africa (WHO, 2004a; UNAIDS, 2004; UNICEF, 2004), is also effective in reducing clinical malaria, independent of baseline CD_4 (Mermin *et al.*, 2004; Anglaret *et al.*, 1999; Chintu *et al.*, 2004). Combined HIV and malaria interventions might best be delivered at peripheral health centers, including antenatal clinics (WHO, 2004a).

HIV-1 appears to have increased the impact of malaria disease and death in South Africa compared to the 1980s, although data do not allow a precise quantification of this effect. In areas of high HIV and low-intensity or unstable malaria, continued vigilance and intensified malaria control are indicated. In HIV-infected adults, pregnant women, and children, malaria is among the simplest opportunistic infections to prevent and treat. Therefore, it is necessary to make use of this advantage presented by the ease in controlling malaria.

2.1.8. WHO RECOMMENDED INTEGRATED APPROACH TO THE DELIVERY OF HEALTH SERVICES RELATING TO MALARIA AND HIV

In order to reduce the lethal consequences of dual infection with HIV and malaria, prevention and treatment programmes of the two diseases must mutually reinforce each other. There is immense potential for synergism, in particular at a time of growing political and financial commitment to reduce the burden of HIV/AIDS, malaria and tuberculosis. The Technical Consultation convened by WHO (WHO, 2004a) agreed on the following key recommendations:

1. As people living with HIV/AIDS in areas of malaria transmission are particularly vulnerable to malaria, their protection by insecticide-treated nets has high priority.
2. HIV-positive pregnant women at risk of malaria should always be protected by insecticide-treated nets and in addition - according to the stage of HIV-infection - receive either intermittent preventive treatment with sulfadoxine-pyrimethamine (at least three doses) or daily co-trimoxazole prophylaxis.
3. Programmes for control of the two diseases should collaborate to ensure integrated service delivery, in particular within the framework of reproductive

health services; and at peripheral health services, where the provision of better diagnostic tools for both diseases, antiretroviral treatment and more effective antimalarial medicines should be undertaken in cooperation.

4. Additional research on interactions between antiretroviral and antimalarial drugs is urgently needed.

2.2 HIV/AIDS

2.2.1 HISTORY OF HIV/AIDS.

Scientists postulate that AIDS may have manifested in humans as far back as 1959. However, it was in 1981 that Kaposi's sarcoma was reported in the New York Times as a rare cancer among gay men in New York and California (Altman, 1981). The same year, emergency rooms in New York City began to receive patients presenting with fevers, flu-like symptoms, and a rare pneumonia called pneumocystis. It became obvious within a short time that heterosexuals, drug addicts, and people who received blood transfusions were also predisposed to the disease. In 1983, researchers at the Pasteur Institute in France and Dr. Robert Gallo, an American, isolated a retrovirus believed and later confirmed to be responsible for the infectious disease. An international committee of scientists renamed the virus HIV (Human Immunodeficiency Virus). A Canadian Flight attendant nicknamed 'patient zero" died of AIDS in 1984. He was believed to have introduced the virus into the general population because of his sexual connection to several of the first victims of AIDS. By that year, there were almost 800 cases which were confirmed in the US, with 3700 deaths (CDC, 1984).

In 1985, Robert Gallo's laboratory patented an HIV test kit that was later approved by the Food and Drugs Administration (FDA). The Pasteur Institute sued and was later awarded rights to half of the royalties from the new test. The same year, a pupil, Ryan White was barred from his elementary school in Indiana, United States of America. Aziathrioprine (AZT) was introduced for the treatment of HIV and AIDS in 1987. After 6 years of watching people die of the disease, AZT was FDA approved and used in high doses. After years of fighting to stay in school and raging an even harder battle against the ravages of HIV, Ryan White died at the age of 19 in 1990. That year, the Ryan White Care Act was enacted by the American congress to provide government sponsored funds for the care of HIV infected people.

In 1992, the American Food and Drugs Administration approved the first drug to be used in combination with zidovudine. The use of this drug, Hovid, marked the beginning of HIV combination therapies. Unfortunately, during this period, three senior French health officials knowingly sold HIV tainted blood, resulting in the infection of hundreds of transfused recipients, most of whom were hemophiliacs. By 1993, people infected and scientists were confused and concerned when a British study, the Concord Trials offered proof that AZT monotherapy did nothing to delay progression to AIDS in asymptomatic patients. This sparked off a period of controversy. In 1996, the protease inhibitors were introduced into the market. The use of the Protease inhibitors in combination with the other drugs already in use resulted in the "triple therapies" gave a new hope. These therapies have been effective. However, reservoirs in the body were discovered and this made the total elimination of the virus impossible(Chun *et al.*,1997;Chun *et al.*, 1998;Finzi *et al.*, 1997). In 1998, the first human trials of an AIDS vaccine began in the United States. In a desperate attempt to get affordable HIV drugs to the hardest hit areas of Africa, European drug

companies ignored the United States patent laws and began making generic versions of HIV medications. In response, drug companies in the United States filed law suits to stop such practices. An African AIDS activist was beaten to death by neighbours after publicly admitting she was HIV positive in the year 2000. Also during that year, the AIDS "re-thinker" movement got international attention and support when South African president Thabo Mbeki questioned the use and effectiveness of HIV medications as well as doubted that HIV degenerated to AIDS. In response, the international scientific community issued the Durban Declaration offering proof that HIV and AIDS were indeed connected.

In the year 2001, scientists became more concerned over medication toxicity and effectiveness. Also, during this year, Médecins sans Frontiers campaigned against the US patent Law, which made the cost of medications unaffordable for the hardest hit areas of sub-Saharan Africa. US pharmaceutical companies dropped their patent law suits, paving the way for European drug companies to manufacture and distribute cheaper HIV medications to these areas in Sub-Saharan Africa. The first entry inhibitor, Fuzeon, was at this time released into the market. Twenty-one million people had died of this disease worldwide including 17 million people from Sub-Saharan Africa. About 60 million people, worldwide, were estimated to have been infected with the HIV, by the year 2002. Also, approximately 20 million people had died of AIDS, which was implied as the leading cause of death in Africa (Kates *et al.*, 2002). By the year 2004, the emphasis on simpler therapies continued, regimen pill burdens were greatly improved with the release of 2 new combination drugs, Truvada and Epzicom as well as two new protease inhibitors, reyataz and lexiva. In December of that year, the first generic formulation of an HIV medication was approved by the FDA the price of HIV medication subsequently reduced.

2.2.2 VIROLOGY OF HIV / AIDS

2.2.2.1 THE EFFECT OF HIV ON THE IMMUNE SYSTEM

AIDS is caused by HIV (Human Immunodeficiency Virus). HIV has a circular shape and it consists of an inner matrix of protein called the core, in which the genetic material (viral RNA) is housed (CDC, 2005). The core is surrounded by an outer layer of protein with numerous small glycoprotein projections on its surface.

Like other viruses, HIV can only reproduce itself inside a living cell. It needs human cells to live in and multiply. However, unlike other viruses, the HIV virus directly attacks and hijacks the most important defensive cells of the human immune system, the CD_4 or the T Helper cells.

As it does this, it slowly diminishes the total number of healthy CD_4 cells in the body thereby undermining the ability of the human immune system to defend itself against attacks from exterior pathogens. When HIV invades the body, the macrophages attempt to do their usual job by engulfing HIV and displaying the antigen. But when CD_4 cells respond to the scene they become infected by HIV, thus starting the attack on the immune system that makes HIV so dangerous to human beings(Geleziunas and Greene ,1999).

The glycoprotein projections on the virus' outer layer attach themselves firmly to the outer layer of the CD_4 cell (onto a CD_4 receptor on the host cell wall).The membranes of the CD_4 cell and virus then fuse. The virus later enters the CD_4 cell with its own genetic material. In order to use the cell to manufacture more viruses, the HIV's viral RNA must be changed (or reverse transcribed) to DNA. The HIV itself carries with it

an enzyme called reverse transcriptase which it then uses to transform its viral RNA into double-strand viral DNA (Geleziunas and Greene, 1999).

.

The viral DNA then fuses with the host cell's own DNA or genetic material in the nucleus of the cell, and makes numerous copies or replicas of the viral RNA and viral proteins. The protease enzymes enable this new viral RNA and the viral proteins to merge and bud from the cell membrane as fully functional HIV viruses (perfect replicas of the original HIV that entered the cell in the first place). As new HIV bud from the cell, they kill the hijacked cell in the process. This does not happen immediately. Each cell can serve as a factory for replicating copies of virus before it finally dies. They then move out into the bloodstream or surrounding tissue to infect more cells and repeat the whole process again. Although all viruses live and multiply solely in cells, the HIV hijacks a critical immune player, the CD_4 cell, and turns it into an efficient virus factory to manufacture and replicate itself. When this happens, the CD_4 cells are unable to do what they would normally do when confronted by an intruder. Although several antibodies are formed during this process, they are ineffective at neutralizing HIV for several reasons. One is that the virus itself has an elaborate outer coating which shields its elements, composed of glycoproteins mentioned above that it uses to break and enter into the cells from the antibodies. Instead, antibodies attach to other shielded parts of the virus which do not stop it invading cells (Geleziunas and Greene, 1999).

Another reason is that HIV is capable of changing its structure to evade immune defenses. There is evidence that the body mounts an antibody response which helps

control HIV earlier on, but that over time, the virus evolves away from these antibodies so that the antibodies are rendered ineffective (Peutherer, 2000).

In a further complication, the CD_8 cells which would ordinarily kill the virus are unable to complete their job. As with antibodies, cellular immune responses to HIV do exist in HIV infected people and there is evidence that they help control the virus, at least for a time. But, in most people, eventually the virus outwits both arms of the immune system. The exact reason for this immune dysfunction is not known. Some explanations include simple exhaustion of the defense cells, and the fact that some regimens, including CD_8 T-cells, are left without orders from the cells which would normally stimulate them to react. They would not be able to mobilize or attack because the T-cells are infected, thus their ability to signal coherent instructions is reduced. It is not only the CD_4 cells that the HIV infects. The glycoprotein projections on the virus' outer layer attach themselves to CD_4 receptors which are present on various types of cells such as monocytes, macrophages, tissue cells in mucous membranes, and certain brain cells. Scientists were initially astonished by the presence of the virus in the brain because the blood-brain barrier usually prevents all foreign substances such as viruses from entering the brain (CDC, 2005).

The knowledge that macrophages are among the few cells that move through the blood-brain barrier, made researchers to quickly deduce that HIV enters the brain by hiding in these very cells (Levy, 2006). The HIV has yet another extraordinary property which allows it to evade the immune system, even when the entire set of defense mechanisms has been mobilized against it. The HIV is able to mutate or change very rapidly. When it copies itself inside a cell, it is literally making copies of its genetic code. Every time the HIV makes an error in this copy, a virus with a

slightly different genetic make-up is produced. Many of these viruses will have no advantage over the usual forms, but some of them will, especially if the error causes changes in the viruses' appearance that help them to evade the immune system. The body's immune system relies heavily on its ability to recognize micro-organisms from their outer protein layer. The body does its best to control the virus and it does, to some extent, which is one reason that people with HIV do not present with illness immediately. But over time, the virus outstrips the immune system, both by killing off T-cells and by constantly changing its appearance to evade fresh products of the immune pathway directed against foreign bodies. This feature of the HIV (the fact that it rapidly changes its identity) is one of the reasons why it is so difficult to develop an HIV vaccine (CDC, 2005).

2.3 DRUG TREATMENT OF HUMAN IMMUNODEFICIENCY VIRUS INFECTION / ACQUIRED IMMUNODEFICIENCY SYNDROME

Antiretroviral drugs are medications for the treatment of infection by retroviruses. These drugs have been used for the treatment and prophylaxis of the Human Immunodeficiency Virus infection. The strategy devised in the use of these drugs presently is to combine 3 or 4 of these drugs in treating patients in order to potentiate the activities of the drugs(AIDS,2005;DHHS,2004). This combination approach is termed highly active antiretroviral therapy or HAART. While all patients presenting with the acquired Immunodeficiency Syndrome require antiretroviral drug therapy, the presence of human immunodeficiency viral infection does not necessarily require antiretroviral drug treatment. The CD_4 count, viral load, symptoms of the patients are taken into account before deciding whether or not the patient will really benefit from antiretroviral drug treatment and therefore taking a decision to place the patient on the

drug (Hammer *et al*., 2008) or to just continue monitoring the patients' status periodically. However, if at any time during the monitoring period there is need for administration of antiretroviral drugs, such patients are placed on the drug.

2.3.1. CLASSIFICATION OF ANTIRETROVIRAL DRUGS USED FOR TREATMENT OF HUMAN IMMUNODEFICIENCY VIRUS:

2.3.1.1. NUCLEOSIDE AND NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS

These act by incorporation into the newly synthesized viral DNA, where they inhibit reverse transcription and prevent its further elongation. Examples of such drugs include: abacavir, emtricitabine, lamivudine, didanosine, zidovudine, apricitibine, elucitabine, racivir, amdoxovir, stavudine, zalcitabine and tenofovir (Deeks and Volberding, 1999).

2.3.1.2. NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS

These drugs inhibit reverse transcriptase directly by binding to the enzyme and interfering with its function. They include efavirenz, nevirapine, etravirine, rilpivirine, loviride, delavirdine (Deeks and Volberding, 1999).

2.3.1.3. PROTEASE INHIBITORS

This group of drugs target viral assembly by inhibiting the activity of protease, an enzyme used by the Human Immunodeficiency virus to cleave nascent proteins for final assembly of new virus. Examples include: saquinavir, lopanavir and ritonavir (Flexner, 2000)

2.3.1.4 . INTEGRASE INHIBITORS

These drugs inhibit the enzyme integrase which is responsible for integration of viral DNA into the DNA of the infected cell. Ralgtegravir is the first among this group to be approved for use in 2007 by the Food and Drugs Administration Department in America though the antiretroviral potential of these drugs had been reported as far back as 1993 (Fesen *et al.*, 1993;Carteau *et al.*,1993).

2.3.1.5. ENTRY INHIBITORS OF FUSION INHIBITOR

These drugs interfere with binding, fusion and entry of Human Immunodeficiency virus- 1 to the host cell by blocking one of several targets. The first to be approved among this group was enfuvirtide (Poveda *et al.*, 2005).

2.3.1.6. MATURATION INHIBITORS

A drug in this class is supposed to inhibit the last step in gag processing in which the viral capsid polyprotein is cleared, thereby blocking the conversion of the poly protein into the mature capsid protein, the virus released consist mainly of non-infectious particles because these viral particles have a defective core. Bevirimat , a drug in this class is still under investigation (Smith *et al.*, 2007).

2.3.1.7. SYNERGISTIC ENHANCERS

These either do not possess antiretroviral properties alone or are inadequate or impractical for monotherapy. However, when they are taken concurrently with antiretroviral drugs they enhance the effect of one or more of those drugs usually by altering the metabolism of these antiretrovirals. These include ritonavir which is an antiretroviral drug that belongs to the class of protease inhibitors. It reduces the hepatic metabolism of other antiretroviral drugs. This principle was first exploited in

the drug Kaletra, which is a combination of ritonavir with the protease inhibitor lopanavir at a ratio (v/v) of 1: 4. Ritonavir is also used as an enhancer of other protease inhibitors such as saquinavir and atazanavir and of the investigational integrase inhibitor, GS 9137(Raffanti and Haas, 2001).

2.3.1.8. VIRAL MUTAGEN

KP-1461 is a newly developed viral mutagen, targeted specifically towards Human Immunodeficiency Virus (HIV) and its sero-types. Its mechanism of action is to induce transcription errors within the Human Immunodeficiency virus during multiplication, ultimately resulting in excessive transcription errors and the death of the virus within immune cells. Presently, it is undergoing clinical trials within the United States. KP-1461 is an oral biochemical precursor of the antiretroviral KP-1212 (Harris *et al.*, 2005). It possesses the following characteristics:

- Low toxicity and it is safe in doses up to and equivalent to 2g/kg body weight in initial model trials.
- Low cross-toxicity with existing antiretroviral drugs currently available in highly active antiretroviral therapy treatment.
- Initial clinical trial has indicated that for certain cohorts the virus is reduced to immeasurable levels.
- Patients with the Human Immunodeficiency Virus who display resistance to other antiretroviral treatments have in all studies thus far responded positively to KP-1461, with significant reduction in viral load (Harris *et al.*, 2005).

Though KP-1461 does not appear to cause short-term mutation in animal models, it is necessary to eliminate this in long-term studies. KP-1461 is not toxic to mitochondria nor does it exbit any inhibitory effect on mitochondrial DNA synthesis (Harris *et al.*, 2005)

2.3.1.9. COMBINATION THERAPY

This is the use of more than one antiretroviral to treat a patient at the same time (AIDS,2005). The life cycle of the Human Immunodeficiency virus can be as short as about 1-5 days from viral entry into a cell, through replication, assembly and release of additional viruses, to infection of other cells (Perelson *et al.*, 1996). The Human Immunodeficiency virus lacks proofreading enzymes to correct errors made when it converts it RNA into DNA via reverse transcriptase. Its short life cycle and high error rate cause the virus to mutate rapidly, resulting in a high genetic variability of the Human Immunodeficiency Virus. Most of the mutations are either inferior to the parent virus often lacking the ability to reproduce at all or convey no advantage, but some of them have a natural selection superior to their parent and can enable them to slip past defenses such as the human immune system and antiretroviral drugs. The more active copies of the virus, the greater the possibility that one resistant to antiviral drugs will be made. Consequently, combination antiretroviral therapy defends against resistance by suppressing the Human Immunodeficiency Virus replication as much as possible. Combination of antiretroviral drugs creates multiple obstacles to the Human Immunodeficiency Virus replication keeping the number of off-spring low and reducing the possibility of a superior mutation (AIDS, 2005). If a mutation arises that conveys resistance to one of the drugs that a patient is on, the other drugs continue to suppress reproduction of that mutation. With rare exceptions, no individual

antiretroviral drug has been demonstrated to suppress a Human Immunodeficiency Virus infection for long. These agents must be taken in combinations in order to have a lasting effect. As a result, the standard care presently is to use combinations of antiretroviral drugs.

Combinations usually comprise two nucleoside analogue reverse transcriptase inhibitor and one non-nucleoside analogue reverse transcriptase inhibitor or protease inhibitor (DHHS, 2004). This three-drug combination is commonly known as a triple cocktail (AIDS, 2005). Combinations of antiretroviral drugs are subject to positive and negative synergies (AIDS, 2005) which limit the number of useful combinations. For example, Didanosine and Zidovudine inhibit each other, so taking them together is less effective than taking either one separately. Other issues further limit some people's treatment options with antiretroviral drug combinations, including their complicated dosing.

2.3.2.0. FIXED DOSE COMBINATIONS

These combinations in fixed doses have been produced to circumvent some of the problems encountered in administering separate drug preparations at the same time. For example, one preparation containing three different drugs can be taken once daily therefore enhancing drug adherence and effectiveness over the long-term. Lack of adherence is usually a primary cause of resistance development to antimicrobials by microbes.Potentiation of drug adherence due to the advent of fixed drug combinations will impact in a positive manner long-term survival currently experienced by patients with HIV (UNAIDS, 2009).

2.3.2.1. CURRENT TREATMENT GUIDELINES

The drug treatment guidelines have changed many times. Earlier, recommendations attempted a "hit hard, hit early" approach. This was followed by a more conservative approach with a starting point somewhere between 350 and 500 CD_4 + T cells/mm3. The current guidelines used new criteria to consider starting highly active antiretroviral therapy. However, there remains a range of views on this subject and the decision of whether or not to commence treatment ultimately rests with the patient and their doctor. The current guidelines for antiretroviral therapy (ART) from the Word Health Organization reflect the 2003 changes to the guidelines and recommend that in resource-limited settings essentially the developing nations, human Immunodeficiency Virus infected adults and adolescents should start antiretroviral therapy when the Human Immunodeficiency Viral infection has been confirmed and one of the following conditions is present (WHO, 2003):

- Clinically advanced Human Immunodeficiency viral disease;
- WHO stage IV Human Immunodeficiency Viral disease, irrespective of the CD_4 cell count;
- WHO stage III disease with consideration of using CD_4 cell counts less than 350/ $\mu\ell$ to assist decision making;
- WHO stage I or II Human Immunodeficiency viral disease with CD_4 cell counts less that 200/$\mu\ell$.

The treatment guidelines for Human Immunodeficiency Virus-I infected adults in the developed world, which comprise countries with access to all or most therapies and

the International AIDS Society-USA (IAS-USA) has set laboratory tests (DHHS, 2008).

The guidelines, set by the International AIDS Society-USA, are developed by a volunteer panel of expert. The recommendation is for therapy to be initiated before CD_4 + cell count declines to below 350 μℓ and to be individualized for the particular patients' situation and co-morbidities (DHHS, 2008). For initial therapy it recommends 2 nucleoside reverse transcriptase inhibitors with either a non-nucleoside reverse transcriptase inhibitor or ritonavir-boosted Protease inhibitor. In antiretroviral therapy failure, the goal of subsequent treatment is suppression of Human Immunodeficiency Virus –I RNA to below detection; the treatment should ideally have 3 new drugs to which the patients' virus is susceptible. This society recommends and sponsors the development of guidelines for the use of drug resistance testing in patients with Human Immunodeficiency Virus – I infection (Hirsch *et al*, 2008).

The treatment guidelines, specifically for the USA, are set by the United States Department of Health and Human Services. The guidelines, which are based on report of symptomatology, CD_4 + T cell count and viral load (DHHS, 2005), state as follows:

- All patients, with a history of an AIDS-defining illness or severe symptom of HIV infection regardless of CD_4+ T cell count, should receive antiretroviral therapy
- Antiretroviral therapy is also recommended for asymptomatic patients with CD_4 + T cell counts of 200 CD_4 +T cells/ μℓ

- Asymptomatic patients with CD_4 +T cell counts of 201 – 350 cells/μℓ should be offered treatment.
- For asymptomatic patients with CD_4+T cell count of greater than 350 cells/ μℓ and plasma HIV RNA greater than 100,000 copies/ml, most experienced clinicians defer therapy but some clinicians may consider initiating treatment.
- Therapy should be deferred for patients with CD_4+T cell counts of greater than 350 cells/μℓ and plasma HIV RNA less than 100,000 copies/ml.

The preferred initial regimens include four groups of drugs (DHHS, 2006)

- efavirenz + zidovudine + lamivudine
- efavirenz + tenofovir + emtricibine
- lopinavir boosted with ritonavir + zidovudine + lamivudine
- lopinavir boosted with ritonavir + tenofovir + emtricibine

In countries with a high rate of baseline resistance, resistance testing is recommended prior to starting treatment, or, if the initiation of treatment is urgent, then empherical treatment regimen likely to be the most active against the virus should be started which is then modified on the basis of resistance testing. In the United Kingdom, there is 11.8% medium to high level resistance at baseline to the combination of efavirenz + zidovudine + lamivudine and 6.4% medium to high resistance to stavudine + lamivudine + nevirapine(UKGTHDR,2005).

Human Immunodeficiency Virus disease in children is more rapid than in adults, and laboratory parameters are less predictive of risks for disease progression, particularly for young infants. Therefore, treatment recommendations from the Department of

Health and Human Services have been more aggressive in children than in adults, in the current guidelines (DHHS, 2005).

2.3.2.2 ANTIRETROVIRAL POST EXPOSURE PROPHYLAXIS AFTER SEXUAL, INJECTION-USE, OR OTHER NON-OCCUPATIONAL EXPOSURES TO HIV IN THE UNITED STATES.

The most effective means of preventing human immunodeficiency virus infection is preventing exposure. The administration of antiretroviral drugs to prevent the human immunodeficiency virus after unanticipated sexual or injection-drug-use exposure might be beneficial. The US Department of Health and Human Services (DHHS) Working Group on Non-occupational post-exposure prophylaxis made the following recommendations for the United States. For persons seeking care ≤ 72 hours after non-occupational exposure to blood, genital secretions or other potentially infectious body fluids of a person known to be HIV infected, when that exposure represents a substantial risk for transmission, a 28-day therapy is recommended. Antiretroviral medications should be initiated as soon as possible after exposure. For persons seeking care ≤ 72 hours after non-occupational exposure to blood, genital secretions, or other potentially infectious body fluids of a person of unknown HIV status, when such exposure would represent a substantial risk for transmission if the source were HIV infected, no recommendations are made for the use post-exposure prophylaxis (DHHS, 2005).

Clinicians should evaluate risks and benefits of post-exposure prophylaxis on a case-by-case basis. For persons with exposure, histories that represent no substantial risk for HIV transmission or who seek care > 72 hours after exposure, DHHS does not recommend the use of post-exposure prophylaxis. Clinicians might consider

prescribing a serious risk for transmission even if the person seeks care > 72 hours after exposure, if in their judgment the diminished potential benefit of post-exposure prophylaxis out-weighs the risks for transmission and adverse events. For all exposures, other health risks, prophylaxis should be administered when indicated. Risk-reduction counseling and indicated intervention services should be provided to reduce the risk for recurrent exposure (Stevens, 2001; Merchant *et al.*, 2002)

2.3.2.3. WHO's RECOMMENDATION FOR POST EXPOSURE PROPHYLAXIS

The World health Organization recommends that the minimum that should be used as HIV post exposure prophylaxis is dual Nucleoside Reverse Transcriptase inhibitors for 28 days with triple therapy where there is a risk of resistance (Moodley *et al.*, 2003). This triple therapy includes dual nucleoside reverse transcriptase inhibitor and a protease inhibitor.

2.3.2.4. PREVENTION OF MOTHER - TO - CHILD TRANSMISSION

Prevention of mother-to-child transmission of HIV-I: where the pregnant woman does not yet need to start antiretroviral therapy for therapeutic reasons, she should start zidovudine from 28 weeks or as soon as possible thereafter be provided with a single-dose nevirapine when entering labour, and be given zidovudine + lamivudine for one week following delivery. Meanwhile, whether the mother was on the above or standard antiretroviral therapy, the child should be given a single-dose nevirapine immediately after delivery and daily zidovudine until one week old (Taha *et al.*, 2003). Complementary measures that may also be used include cesarean section and formula feeding, in some settings, the combination of providing all these measures

has succeeded in reducing the risk of infection from 25% to about 1% (Wiktor *et al*., 1999).

2.4 MALARIA

2.4.1 HISTORY OF MALARIA

Malaria is probably one of the oldest diseases known to mankind. Man and Malaria seem to have evolved together and it has been known to mankind for millennia (Pennisi, 2001; Tishkoff *et al*., 2001). It was always part of the ups and downs of nations, of wars, and of upheavals. Mentions of this disease can be found in the ancient Chinese, Indian and Egyptian manuscripts. Charaka and Sushrutha, the two leading lights of the Indian system of medicine, "Ayurveda," gave vivid descriptions of malaria and even associated it with the bites of the mosquitoes. The aborigines of Peru and Ecuador used the tincture of the cinchona bark for treating fevers before the seventeenth century, while Huan del Vego employed this herb for treating malaria in 1640. Morton, in 1696, presented the first detailed description of the clinical picture of malaria and treatment with cinchona.

Lancisi in 1717 linked malaria with poisonous vapours of swamps and thus originated the name malaria, meaning bad air. In 1816, Gize studied extraction of crystalline quinine from the cinchona bark and in 1820, Pelletier and Caventou extracted pure quinine alkaloids. Laveran, a French physician working in Algeria, identified the causative agent for human malaria in 1880 while viewing a slide of blood under the microscope (Russel *et al*., 1951). He won the Nobel Prize in 1907. In the 1800s, there was a decline in the incidence of Malaria in Northern Europe and the United States because swamps were drained for agricultural use and there was better housing and

screening. However, it was also estimated that 50% of the white soldiers and 80% of the black soldiers got infected with malaria annually during the American Civil War of 1861 – 1865.

The mosquito transmission hypothesis for malaria was first put forward in 1882. Golgi identified *P.vivax* and *P.malariae* in 1885, while Sakharov in 1889 and Marchiafava and Celli in 1890 identified *P. falciparum* (Short *et al.*, 1949; Short *et al.*, 1951). Romanowsky described staining methods for identifying malaria parasites in 1891. During the same year, Ehlirch discovered the mild antimalarial activity of methylene blue. In 1894, Manson hypothesized that mosquitoes transmitted malaria. In 1897, while working as a military physician in India, Ronald Ross demonstrated the malaria oocysts in the gut tissues of the female Anopheles mosquito, thus proving the fact that Anopheles mosquitoes were the vectors for malaria. Ross was awarded the Nobel Prize in 1902. Giovanni Batista Grassi proved that malaria was transmitted by the anopheles mosquito to people in 1898.

Scientists in Germany, during the World War I, developed the antimalarial drug atebrine. Mietzsch, Mauss and Hecht were notable researchers. J. Wagner Von Jauregg, in 1927, received the Nobel Prize in Medicine for his work in treating syphilis using malaria parasites. Patients were inoculated with the malaria pathogen from which three or four bouts of fever, resulting from this infection, were enough to burn up the temperature sensitive syphilis bacteria (*Treponema pallidium*). Once cured of syphilis, the patient was given quinine to get rid of the malaria. This treatment of syphilis was replaced in the 1950s by the use of antibiotics. In 1928, Schuleman, Schonhofer and Wingler synthesized plasmoquine. Knunyants and Chelintsev synthesized acriquine in 1930. A German company developed Resochin in

1934. In 1939, Paul Muller discovered the insecticidal properties of DDT, a drug which has been useful over the years for eradicating mosquitoes (Gilles, 2002)

In the 1940s, there was large scale spraying of the breeding areas of mosquitoes with DDT in the United States. This programme was successful in the US, Brazil and Egypt. Research into antimalarial drugs was intensified during World War II and the 4-amino quinoline, initially produced as Resochin by the Germans, was slightly modified and presented as chloroquine by the Americans. Subsequently, the 4-amino quinoline played a prominent role in the therapy of malaria for many decades. In 1948, Short, Garnham, Covell and Shute identified the tissue forms of *P. vivax* in the liver. The tissue stages of *P. falciparium* (Short *et. al.*, 1949), *P. ovale* and *P. malariae* were also identified later. In the early 1950s, DDT resistance by Anopheles developed in several countries, prompting renewed efforts to use it on a wide scale before the resistance became too widespread. Elderfield of USA synthesized primaquine in 1950. Graham identified the tissue form of *P. ovale* in 1954 and that of *P. malariae* was identified by Bray in 1959. The World Health Assembly launched the malaria eradication initiative in 1955. Africa was excluded because of the extent of malaria transmission and because of lack of infrastructure. The goal was to reduce infected vector populations feeding on humans sufficient to interrupt parasite transmission, rather than trying to eradicate all vectors. Malaria was eradicated in the temperate zones but in places like Sri Lanka, initial successes were followed by resurgences of the disease.

There was the appearance of *Plasmodium falciparum* strains resistant to chloroquine from 1960 in South America and South East Asia, spreading to most parts of the

world by the 1970s (Baird, 2000). In the 1970's, resources for malaria control shifted largely to other health needs, and the DDT was removed from the market in the United States because of its environmental effects. Alternative insecticides which were more expensive replaced the DDT. Some of these agents were even more toxic to humans.

Between 1969 and 1976, the World Health Organization coordinated an intensive study of malaria in the Garki district of Northern Nigeria. This study highlighted the problems that could be encountered in combating malaria in Africa. These problems included high bite intensity, high proportion of vectors carrying the parasite, mosquitoes resting outdoors after blood meals instead of indoors on insecticide treated walls. It was concluded that the use of drugs and insecticides could markedly reduce the incidence of malaria in the short term but was not enough to break the transmission and achieve long-term success (WHO,2008).

By 1980, chloroquine resistance by *P.falciparum* appeared in coastal Tanzania and Kenya and subsequently spread over most of Africa. In 1982, Bray and Garnham proposed that some sporozoites in the liver remain latent (hypnozoites), causing relapses later on. In the 1980s, the use of the combination drug, sulphadoxine - pyrimethamine in the treatment of malaria gained grounds because it was an alternative in the treatment of resistant strains and it was easy to administer since it required just one dose. The use of quinine in the treatment of severe or resistant *P. falciparum* infection also became a standard. In 1987, Dr. Manuel Elkin Patarroya, a Colombian biochemist, developed the first synthetic vaccine against the *Plasmodium falciparum* parasite. In the early 1990s, pockets of regions, with *Plasmodium*

falciparum resistant to the sulphadoxine-pyrimethamine combination, were confirmed. Subsequently, there has been an increase in the surge of this resistance (Roper, 2003). The research into newer drugs to combat resistance strains led to the discovery of Halofantrin in the 1990s and it was a very effective therapy. Also, combination therapies employing sulphadoxine / pyrimethamine with chloroqine, chloroquine with tetracycline, chloroquine with co-trimoxazole became popular in the management of resistant strains.

In addition, amodiaquine and mefloquine played prominent roles in the management of resistant strains during this period. Eventually, *P. falciparum* developed resistance to virtually all drugs available. By the close of the millennium, the issues of drug resistance, morbidity and mortality due to malaria were extremely alarming, including the impact on pregnant women and children, particularly those less than 5 years of age. This has resulted in new emphasis on the scourge of malaria globally and attempts at achieving an effective control, employing among others new drug combinations and vector-directed barriers / eradication such as the insecticide treated nets (WHO, 2008; Sirima *et al*., 2003; Jones *et al*., 2003; Takken, 2002**)**. Artemisimin based combination therapy is the standard treatment for malaria fever, although the combination of Atovaquone / proguanil has also been employed and found to be useful. A lot of work still needs to be done in view of the constant emerging resistance to drugs, insecticides and socio-economic factors (Phillips, 2001).

2.4.2 CLINICAL FEATURES OF MALARIA

Fever is a prominent feature. Prodromal symptoms consist of malaise, muscle pains, headache, anorexia, joint pain, dizziness, lassitude, and tiredness. Slight fever may exist for a few days before the onset of the acute phenomena. At other times, the acute

attack starts off either without prodromal symptoms or with prodromal features which are not so noticeable by the patient .The acute phase symptoms include moderate to sever fever associated with chills and rigors, headache, nausea, vomiting, diarrhoea, constipation, weakness, abdominal pain, moderate body pain. Upon presentation, the suggestive factor is the history of domicile in a mosquito - infested country, and fever is the cardinal sign which the clinician should always verify with the use of a thermometer.The pattern of the fever should be ascertained from the patient for the purpose of differentiating it from other possible causes such as viral infections and bacterial infections, particularly typhoid fever in the tropics. Also, knowledge of the pattern of fever may be useful in determining the specie of plasmodium which has infected the patient. The patient may manifest with complications therefore presenting with jaundice, anaemia, haematuria, spleenomegaly, hepatomegaly, alteration of the level of consciousness, which may vary from a delirious state to coma, and alteration of the muscle tone when cerebral malaria ensues (Rathbun, 2000).

2.4.3 PATHOGENESIS OF MALARIA

Infection in human is caused by *P. falciparum*, *P. Ovale*, *P. malariae*, and *P. vivax* which are obligate intracellular protozoa (Tracy and Webster, 2001). Although transmission can be by transfusion of infected blood and sharing of needles, it is mostly by the bite of infected female mosquitoes of the genus Anopheles. When the mosquito bites an exposed person, it releases the sporozoites into the victim's blood. These get into the liver where it undergoes transformation and multiplication, developing into tissue Schizonts. This pre or exoerythrocytic stage lasts between 5 to 15 days depending on the Plasmodium species. Subsequently, the tissue Schizonts rupture, and are released into the blood circulation as merozoites which invade the

erythrocytes, this is the erythrocytic stage. In the red blood cell**,** they undergo asexual development from young ring forms to trophozoites and finally, matured Schizonts. Red blood cells containing Schizonts rupture releasing 6-24 merozoites. This process, which is called Schizogony, causes the febrile illness. The merozoites invade more red blood cells and this continues till modulation by drugs, acquired partial immunity or death of host. Schizogony is about 48 hours in *P. falciparum, P. vivax P.ovale*, therefore, febrile illness attacks occur on days 1 and 3, hence they are described as tertian malaria while in *P. malariae*, it occurs every 72 hours and infection by this species is termed quartian malaria. Once the tissue Schizonts burst in the *P. falciparum* and *P. malariae*, no parasite remains in the liver. In the *P. ovale* and *P. vivax*, however, forms remain which can cause relapses months to years after the primary attack (Cogswell, 1992). Also, once the Plasmodia set in the erythrocytes, they cannot invade tissues; hence there is never any tissue form when malaria is acquired by blood transfusion.

2.4.4 LABORATORY DIAGNOSIS

This has been done over the years by demonstrating the presence of the parasite in the blood smear of the patient made on a glass slide. A thick film is more sensitive in detecting the presence of the parasite, and also saves time in examination while the thin film technique causes very little distortion of the parasite, and permits species' identification when it may not be possible in the thick film. Many fields may, however, have to be examined to detect the parasites when they are few in number. The thin film is fixed with methanol, and the two different types of films are stained with Giemsa, one of the Romanowsky stains. The slide is then examined under a light microscope (Greenwood, 2000).

Rapid diagnostic tests for malaria have recently emerged. Some of these are specific for particular species of plasmodium, while others can detect all species. They are immunological tests which detect the presence of antigens in the plasmodia or antigenic substances released from the infected red blood cell. A blood specimen from the infected patient as well as a reagent is added to a test strip. After some minutes, indicators on the strip reveal the presence or absence of plasmodium infection. Though these tests may not be as reliable as the observation of the parasite on thin and thick blood films, the sensitivity of some kits such as the Kat-Quick Malaria test for *P. falciparium* is as high as 96% and specificity up to 99.7%. This particular version detects the presence of a specific soluble antigenic protein, histidine-rich protein II (pFHRP-II), which is present and released from red blood cells infected with *P. falciparium* (Rathbun ,2000).

2.4.5 DRUGS USED IN THE TREATMENT OF MALARIA

2.4.5.1 CLASSIFICATION OF ANTIMALARIA DRUGS

Antimalarial drugs can be classified according to antimalarial activity and structure.

1. According to antimalarial activity,

   a. Tissue schizonticides for causal prophylaxis: These drugs act on the primary tissue forms of the plasmodia which after growth within the liver, initiate the erythrocytic stage. By blocking this stage, further development of the infection can be theoretically prevented. Pyrimethamine and Primaquine have this activity. However since it is impossible to predict the infection before clinical symptoms begins; this mode of therapy is more theoretical than practical.

b. Tissue schizonticides for preventing relapse: These drugs act on the hypnozoites of *P. vivax* and *P. ovale* in the liver that cause relapse of symptoms on reactivation. Primaquine is the prototype drug; pyrimethamine also has such activity (Tracy and Webster,2001).

c. Blood schizonticides: These drugs act on the blood forms of the parasite and thereby terminate clinical attacks of malaria. These are the most important drugs in antimalarial chemotherapy. These include drugs such as chloroquine, quinine, mefloquine, halofantrine, pyrimethamine, sulfadoxine, sulfones and tetracyclines.

d. Gametocytocides: These drugs destroy the sexual forms of the parasite in the blood and thereby prevent transmission of the infection to the mosquito. Chloroquine and quinine have gametocytocidal activity against *P. vivax* and *P. malariae*, but not against *P. falciparum*. Primaquine has gametocytocidal activity against all plasmodia, including *P. falciparum*.

e. Sporontocides: These drugs prevent the development of oocysts in the mosquito and thus ablate the transmission. Primaquine and chloroguanide have this action (Tracy and Webster, 2001).

2. <u>According to the structure:</u>

- **Aryl amino alcohols:**

  Quinine, quinidine (cinchona alkaloids), mefloquine, halofantrine.

- **4-aminoquinolines:**

  Chloroquine, amodiaquine.

- **Folate synthesis inhibitors**:

Type 1 –These are employed primarily as antibacterial but have also been useful in malaria therapy (Petri, 2001). Drugs under this class include competitive inhibitors of dihydropteroate synthase - sulphones,

sulphonamides

Type 2 – Drugs that inhibit dihydrofolate reductase - biguanides like proguanil and chloroproguanil; diaminopyrimidine like pyrimethamine

- **8-aminoquinolines:**

  Primaquine, WR238, 605

- **Anti-bacterials:**

  Tetracycline, doxycycline, clindamycin, azithromycin, fluoroquinolones

- **Peroxides:**

Artemisinin (Qinghaosu) derivatives and analogues - artemether, arteether, artesunate, artelinic acid

- **Naphthoquinones:**

  Atovaquone

- **Iron chelating agents:**

  Desferrioxamine

The structures of these antimalaria drugs are illustrated in figures 1 and 2 (Tracy and Webster, 2001).

FIGURE 1:

FIGURE 2:

2.4.5.1.1 CHLOROQUINE AND ITS CONGENERS

- **History**

Chloroquine is one of a large series of 4-aminoquineolines investigated in the United States during World War II. The objective was to discover more effective and less toxic suppressive agents than quinacrine. Although the 4-aminoquinolines had previously been described as potential antimalarials by Russian investigators, serious attention was not paid to this chemical class until the French reported that 3-methyl-7-chloro-(4-diethylamino-1-methylylamino) quinline (SN-6911; SONTOCHIN, SONTOQUIN) was well tolerated and had high activity in human malaria infection. Beginning in 1943, thousands of these compounds were synthesized and tested for activity in avian malaria and for toxicity in mammals, ten of the series were then examined in human volunteers with experimentally induced malarias. Of these, chloroquine proved most promising and was released for field trial. When hostilities ceased, it was discovered that the chemical had been synthesized and studied under the name Resochin by the Germans as early as 1934(Gilles, 2002).

- **Chemistry**

Chloroquine disphophate is a white, bitter powder, soluble in water. Its solutions are stable. Structure-activity relationship indicates that chloroquine contains the same alkyl side chain as quinacrine. It differs from quinacrine in having a quinoline instead of an acridine nucleus and in lacking the methoxy moiety. Chloroquine also bears close resemblance to pamaquine and pentaquine. The 4-aminoquinolines showing the most marked antimalarial activity in both avian and human malarias have a chlorine

atom in position 7 of the quinoline. Methyl substitution in position 3 of the quinoline reduces activity, and additional methyl substitution in position 8 completely eliminates activity (Beliner *et al.*, 1948, Coatney *et al.*, 1953)

Amodiaquine is a congener of chloroquine. Currently, it is employed in combination with the Artemisinin derivatives for treatment of malaria fever. The properties of Amodiaquine and dosage are similar to those of chloroquine. Hydroxycloroquine, a compound in which one of the N-ethyl substituents of chloroquine is B-hydroxylated is preffered over chloroquine for treatment of mild rheumatoid arthritis and lupus erythematosis because, given in the high doses required, it may cause less ocular toxicity than chloroquine would (Easterbrook, 1999).

- **Pharmacological effects**:

Although chloroquine was developed primarily as an antimalarial agent, it possesses several other pharmacological properties. It is used to treat extra intestinal amebiasis. Chloroquine and hydroxychloroquine have been used as secondary drugs to treat a variety of chronic diseases because both alkaloids concentrate in lysosomes and have anti-inflamatory properties. These include rheumatoid arthritis, systemic lupus erythematosus, discoid lupus, sarcoidosis and photosensitivity diseases such as porphyria cutanea tarda and severe polymorphous light eruption (Danning and Bonmpas,1998; Fritsch *et al.*, 1998;Baltzan *et al.*,1999; Dubois, 1978)

Treatment of these conditions requires much larger doses than are used for malaria, and this mandates proper consideration of the toxicity of this agent (Isaacson *et al.*, 1982).

- **Antimalaria Actions**:

Chloroquine, even in massive doses, exerts no significant activity against the exoerythrocytic tissue stages of plasmodia. The drug is thus not a causal prophylactic agent and does not prevent the establishment of infection. Chloroquine is effective against the asexual erythrocytic forms of *P .vivax* and *P. falciparium*, and gametocytes of *P. vivax*, although its ability to eliminate these species of plasmodia has decreased considerably over the years because of formation of resistant strains especially in the case of *P. falciparium*. Chloroquine, like quinine, does not prevent relapses in vivax malaria, but it substantially lengthens the interval between relapses. Chloroquine is well tolerated and is thus easier to administer than quinine.

Mechanism of Action:

From early work, it has been hypothesized that chloroquine might exert its effect, at least in part by an interaction with DNA. Chloroquine inhibits the incorporation of 32P labeled phosphate into RNA and DNA by *P.gallinaceum in vitro* and *in vivo*, and by *P.berghei in vitro* (Schellenberg and Coatney, 1960). Chloroquine combines strongly with double stranded DNA. The drug inhibits DNA and RNA polymerase by combining with DNA primer (Allison *et al.*, 1965; Cohen and Yielding, 1965). Changes in several physical parameters were consistent with an intercalation of chloroquine with guanine containing double stranded DNA (Allison *et al.*, 1966) Such intercalation also occurs with primaquine and quinine, but not with mefloquine, an antimalarial structurally related to quinine (Davidson *et. al.*, 1977). Failure to demonstrate intercalation in the case of mefloquine however does not rule out other types of intercalations of these antimalarials with DNA. Plasmodium-infected erythrocytes exposed to chloroquine concentrate the drug and also exhibit clumping

of malaria pigment that forms as the parasite digests the hemoglobin of the host red cells. The two processes may be related, in that both are energy dependent, saturable, and competitively inhibited by antimalarials such as amodiaquine, quinine, and mefloquine (Chou *et al.*, 1980). Aggregates of ferriprotoporphyrin IX, released during degradation of hemoglobin by parasitized erythrocytes, may serve as a receptor for chloroquine and related antimalarial compounds and thus account for accumulation of the drug. Either ferriprotoporphyrin IX or complexes of chloroquine with the porphyrin can cause membrane damage with lysis of trypanosomes, erythrocytes, or malarial parasites (Meshnick *et. al.*, 1977; Dutta and Fitch, 1983; Fitch, 1983).

- **Pharmacokinetics**:

Chloroquine is deposited in the tissues in considerable amounts. In animals, from 200 to 700 times the plasma concentration may be found in the liver, spleen, kidney, lung and melanin-containing tissues; leukocytes also concentrate the drug. The brain and spinal cord in contrast, contain only 10-30 times the concentration present in plasma. Chloroquine undergoes appreciable biotransformation. The main metabolite is desethylchloroquine, which accounts for one fourth of the total material appearing in the urine, bisdesethyl chloroquine, a carboxylic acid derivative and other uncharaterised metabolites are found in small amounts. Slightly more than half of the urinary drug products can be accounted for as unchagnged chloroquine (Tracy and Webster, 2001).

Desethylation to the secondary amine results in a substance that is highly active against avian malaria. Metabolic products of chloroquine may thus be partially responsible for antimalaria activity. For effective plasma concentrations to be

achieved and maintained, a loading dose is essential because of the avidity of tissues for the drug. When the drug is discontinued after daily dosage for 2 weeks, plasma concentrations and urinary excretion both decrease with a half-life of 6 to 7 days for the next 4 weeks; subsequently the half-life for urinary excretion increases to about 17 days. Small amounts can be found in the urine for several years. Daily oral dosage of 310 mg of chloroquine base results in a steady-state concentration in plasma of about 125 µg per litre (Rollo,1980).

With a weekly 0.5-g dose, the peak concentration in plasma varies between 150 and 250µg per litre; just prior to the succeeding dose, the ranges is between 20 and 40 µg per litre. This compares to therapeutic concentrations of about 30 µg per litre for drug-sensitive *P. falciparum* and 15 µg per litre for *P. vivax*. After single or weekly doses, the half-life of the drug in plasma is about 3 days. Congeners of chloroquine (such as amodiaquine) interfere with its metabolism with their concurrent use, plasma concentrations of chloroquine are elevated for prolonged periods (Rollo,1980).

- **Preparations**:

Chloroquine phosphate is available as tablets containing either 250 or 500 mg of the diphosphate. Approximately 60% of the diphosphate represents the base. Chloroquine hydrochloride is available as an injection (50 mg/ml; equivalent to 40 mg/ml of the base). It is also combined in tablets with primaquine for prophylactic use only. Hydroxychloroquine sulphate is available in 200-mg tablets, equivalent to 150 mg of the base.

Chloroquine phosphate is given orally in tablet form; either before or after meals. The hydrochloride salt of chloroquine may be employed for parenteral (intramuscular) injection when necessary. For the purpose of suppressive therapy, an oral dose of 500 mg of the phosphate is given to adults on the same day of each week starting 1 week before and continuing for at least 6 weeks after the last exposure in an endemic area. Usual pediatric doses are 5 mg/kg of the base weekly (Rathbun, 2000).

In many countries, chloroquine is no longer the drug of choice for treatment of *P. falciparum* because of development of resistance to this drug which had been very useful for many decades. Artemisinin based combination therapy and Atovaquoue-proquanil combination are now used more commonly. For treatment of the acute attack of vivax or chloroquine sensitive-strains of *P. falciparum*, an initial loading dose of 600mg of chloroquine base in administered; followed by an additional 300mg after 6 or 8 hours and a dose of 300 mg on each of 2 consecutive days (Rollo, 1980).

- **Toxicity and side Effects**:

Chloroquine may cause gastrointestinal upset, pururitus, mild and transient headache and visual disturbances. Prolonged chronic medication for suppressive purposes causes few significant untoward effects, and rarely has the drug been discontinued because of intolerance. All symptoms readily disappear when the drug is withheld. Chloroquine may cause discoloration of nail beds and mucous membranes. Prolonged treatment with chloroquine may cause a lichenoid skin eruption in a few patients; the

condition is mild and subsides promptly when the drug is discontinued. Readministration of chloroquine usually does not result in reappearance of the lesion.

Large doses given for a year to a group of healthy volunteers occasionally caused some visual symptoms which include blurring of vision, diplopia, bleaching of the hair, T wave abnormalities in the ECG, mild skin eruptions, headache, and slight weight loss; the observed toxic effects caused no incapacity and were reversible upon withdrawal of the drug (Alving *et al.*, 1948). High daily doses greater than 250 mg of chloroquine, used for long-term treatment of diseases other than malaria, can result in irreversible retinopathy. This complication, presumably related to deposition of drug in melanin – rich tissues (Bernstein *et al.*, 1963), can be avoided if the daily dose is 250 mg or less (Dubois, 1978; Olansky, 1982). Rarely, neuropsychiatric disturbances, including unintentional suicide, may be related to over dosage (Good and Shader, 1982). There is no convincing evidence that chloroquine given during pregnancy causes fetal abnormalities.

Precautions and contraindications:

Chloroquine should be used with caution in the presence of hepatic disease because of the high concentration that occurs in the liver. It should be used cautiously or not at all in the presence of severe gastrointestinal, neurological, or blood disorder. If such disorders occur during the course of therapy, the drug should be discontinued. Concomittant use of gold or phenylbutazone with chloroquine should be avoided because of the tendency of all three agents to produce dermatitis. For patients on long

term, large dose therapy, ophthalmological examination is recommended before and periodically during treatment (Good and Shader, 1982).

2.4.5.1.2. CHLOROGUANIDE AND CYCLOGUANIC PAMOATE

Chloroguanide is known internationally as proguanil. It is a biguanide derivative that emerged as a product of British antimalaria research during World War II. In mammals, the compound is converted to a triazine metabolite that acts as a blood schizontocide by inhibiting plasmodial dihydrofolate reductase. Chloroguanide has been used primarily for long-term prophylaxis and suppression of chloroquine-sensitive strains of *P. falciparum*. Unfortunately, however, its clinical effectiveness is greatly compromised by the presence of rapid development of drug-resistant strains of *P. falciparum*. Nonetheless, chloroguide represented an important advance because it opened the field for the development of other antifolates (Tracy and Webster,2001).

2.4.5.1.3 DIAMINOPYRIMIDINES

Pyrimethamine was developed and used almost solely as an antimalarial agent.

Trimethoprim was created as an antibacterial agent and later found to have antimalarial properties. Pyrimethamine was found to be highly effective against the plasmodia infecting man (Falco *et. al.*, 1951) and was used widely for prophylaxis and suppression.

- **Pharmacological Effects: Antimalarial Actions and Efficacy**

The antimalarial effects of pyrimethamine are similar to those of chloroguanide. It acts directly and the half-life is much longer than that of the active metabolite of chloroguanide. The major use of pyrimethamine was in prophylaxis, suppression, and combined chemotherapy of chloroquine resistant strains to *falciparum* malaria. At therapeutic doses, pyrimethamine fails to eradicate latent tissue forms of *P. vivax* or gametocytes of any plasmodial species (Tracy and Webster ,2001)

- **Mechanisms Of Antimalaria Action:**

In an elegant series of investigations, the 2, 4-diaminopyrimidines were shown to act by inhibiting dilydrofolate reducatase of plasmodia at concentrations far lower than required to produce comparable inhibition of the mammalian enzymes (Ferone *et al.*, 1969) Dihydrofolate reductase catalyzes the reduction of dihydrofolate to tetrahydrofolate, which is in turn required for the biosynthesis of purines, pyrimidines, and certain amino acids. Inhibition of dihydrofolate reductase by pyrimethamine is manifested in the malarial parasite by failure of nuclear division at the time of schizont formation in erythrocytes and liver (Ferone *et al.*, 1969).

The concept of inhibiting two steps in an essential metabolic pathway with separate drugs to produce a supra-additive effect explains the synergistic action of the 2, 4-diaminopyrimidines with the sulfonamides or sulfones (Hitchings and Burchall, 1965). The two steps involved are the utilization of para-aminobenzoic acid (PABA) in the synthesis of dihydropteroic acid, inhibited by sulfonamides, and the reduction of dihydrofolate to tetrahydrofolate, inhibited by pyrimethamine. About one eight of the ED50 of pyrimethamine and sulfadiazine administered together was equivalent to the ED50 of either used alone in experimental malarial infections (Rollo, 1955). Hurly

(1959) treated African children infected with *P. falciparum* and *P. malariae* with pyrimethamine and sulfadiazine, alone and in combination; clinical cure was obtained with the combination of less than one tenth of the curative dose of pyrimethamine plus less than one fourth of the curative dose of sulfadiazine. Subsequently, trials of several combinations of pyrimethamine and either sulfonamides or dapsone confirmed the augmentative effect both in the suppression and in the treatment of acute falciparium infections (Donno *et al.*, 1969; Lucas *et al.*, 1969). The value of such combinations lies in preventing or delaying strains of plasmodia from developing resistance to these drugs. Such strains have arisen readily when small doses of pyrimethamine alone were used for long periods of time. Suitable combinations have shown their value in the treatment and suppression of some multiresistant strains (WHO,1981). Combinations of trimethoprim, the related diaminopyrimidine, with sulfamethoxazole were of particular value in the treatment of bacteria infections towards the end of the last mellinium.

- **Pharmacokinetics:**

Pyrimethamine is slowly but completely absorbed after oral administration. The compound accumulates mainly in the kidneys, lungs, liver, and spleen and is eliminated slowly with a half-life in plasma of about 4 days. Concentrations that are suppressive for drug-sensitive strains remain in the blood for 2 weeks (Brooks *et al.*, 1969; Stickney *et al.*, 1973). Several metabolites of pyrimethamine appear in the urine. Pyrimethamine is also excreted in the milk nursing mothers.

- **Preparations, route of administration, and dosage**:

It is marketed as Daraprim in 25mg strength tablet with sulphadoxine 25mg/500mg as Fansidar. It is possibly the most frequently used antimalaria combination, taken as sulphadoxine /pyrimethamine, 1500/75mg stat adult dose. It is now recommended for Intermittent Preventive Therapy of malaria in pregnant women living in malaria endemic regions and combined with Artemisinin for cure in Nigeria.

2.4.5.1.4 SULFONAMIDES

- **History**

The sulfonamide drugs were the first effective chemotherapeutic agents to be employed systemically for the prevention and cure of bacterial infectious in human beings. The considerable medical and public health importance of their discovery and their subsequent widespread use were quickly reflected in the sharp decline in morbidity and mortality figures for the treatable infectious disease. The advent of penicillin and subsequently of other antibiotics has diminished the usefulness of the sulfonamides, and they presently occupy a relatively small place in the therapeutic armamentarium of the physician (Petri, 2001).

However, the introduction in the mid-1970s of the combination of trimethoprim and sulfomethoxazole has resulted in the increased use of sulfonamides for the treatment of specific microbial infections. Investigations at the I.G. Farbenindustrie resulted, in 1932, in a German patent to Klarer and Mietzsch, covering PRONTOSIL and several other azo dyes containing a sulfonamide group. Domagk, a research director of the I.G. working with Klarer and Mietzsch, was aware of the fact that synthetic azo dyes had been studied for their action against streptococci, which prompted him to test the

new compounds. He quickly observed that mice with streptococcal and other infections could be protected by PRONTOSIL (Domagk, 1935). Domagk received the Nobel Prize in Medicine for 1938, a credit for the discovery of the chemotherapeutic value of PRONTOSIL. Foerster reported the first clinical case on a 10 month-old infant in 1933. Also, favourable clinical results with PRONTOSIL and its active metabolite, sulfanilamide, in puerperal sepsis and meningococcal infectious were later reported (Colebrook and Kenny, 1936; Buttle *et al*., 1936).

- **Chemistry**

Sulfonamide is the generic name for derivatives of para – aminobenzenesulfonamide (Sulfanilamide). Most of them are relatively insoluble in water but their sodium salts are readily soluble. The minimal structural prerequisites for antibacterial action are all embodied in sulfanilamide itself. The-SO_2NH_2 group is not essential as such, but the important feature is that the sulfur is directly linked to the benzene ring. The para-NH_2 group is essential and can be replaced only by such radicals that can be converted *in vivo* to a free amino group. However, substitution of heterocyclic aromatic nuclei at N^1 yields highly potent compounds. The more negative the SO_2 group of an N^1-substituted sulfonamide, the greater is the bacteriostatic activity (Bell and Robin, 1942). Optimal activity had thus been achieved in sulfadiazine (Bell and Robin, 1942)..

- **Antibacterial Spectrum.**

a. Effects on microbial organisms. Sulfonamides have a wide range of antimicrobial activity against both gram-positive and gram-negative microorganisms with a few exceptions. There is direct correlation between their

efficacy *in vitro* and *in vivo*. In general, the sulfonamides exert only bacteriostatic effect in the body, and cellular and humoral defence mechanisms of the host are essential for the final eradication of the infection.

b. Synergists and Antagonists of sulfonamides. Trimethoprim exerts a synergistic effect when used with a sulfonamide (Bishlay and Hit-Chings, 1968). The compound is a potent and selective competitive inhibitor of microbial dilydrofolate reductase, the enzyme that reduces dihydrofolate to tetrahydrofolate. PABA is the most prominent among the sulfonamide antagonists. Certain local anesthetics, such as procaine, that are esters of PABA antagonize these drugs *in vitro* and *in vivo*. PABA may be added to cultures of blood or body fluids in order to block the inhibitory effect of sulfonamides on microbial growth, sensitivity to sulfonamides must be determined in media that are free of PABA. The antibacterial action of this drug is also inhibited by blood, pus, and tissue breakdown products because the bacteria requirement for folic acid is reduced in media that contain purines and thymidine (Mandell and Sande, 1980).

c. Effects of sulfonamides combined with other chemotherapeutic agents. Investigations of the activity of combinations of sulfonamides and antibiotics *in vitro* and in experimental animals suggest an additive effect when sulfonamide is combined with bacteriostatic agents such as the tetracyclines and either an antagonistic or a synergistic effect when bacteria are exposed simultaneously to sulfonamides and a bacterial antibiotic.

d. Acquired Bacterial Resistance to sulfonamides. Bacteria, initially sensitive to sulfonamides, can acquire resistance to the drug both *in vitro* and *in vivo*.

Bacteria resistance to sulfonamides originate by random mutation and selection or by transfer of resistance by plasmids. Such resistance, once it is maximally developed, is usually persistent and irreversible, particularly when produced *in vivo*. Acquired resistance to sulfonamide usually does not imply cross – resistance to chemotherapeutic agents of other classes. The *in- vivo* acquisition of resistance has little or no effect either on virulence or on antigenic characteristics of microorganisms. Resistance to sulfonamide is probably the consequence of an altered enzymatic constitution of the bacterial cell; the alteration may be characterized by (1) an alteration in the enzyme that utilizes PABA, dihydropteroate syntheses; (2) an increased capacity to destroy or inactivate the drug; (3) an alternative metabolic pathway for synthesis of an essential metabolite; or (4) an increased production of an essential metabolite or drug antagonist. The latter possibility has received most attention. For example, some resistant staphylococci may synthesize 70 times as much PABA as do the susceptible parent strains. Nevertheless, an increased production of PABA is not a constant finding in sulfonamide-resistant bacteria, and resistant mutants may possess enzymes for folate brosynthesis that are less readily inhibited by sulfonamides (Mandell and Sande, 2001).

- **Mechanism of Action.**

Sulfonamides are structural analogs and competitive antagonists of para-aminobenzoic acid (PABA), and thus prevent normal bacterial utilization of PABA for the synthesis of pteroylglutamic acid (Fildes, 1940; Woods, 1940). Sulfonamides are competitive inhibitors of the bacterial enzyme responsible for the incorporation of PABA into dihydropteroic acid, the immediate precursor of folic acid. Sensitive

microorganisms are those that must synthesize their own PGA; bacteria that can utilize preformed PGA are not affected. Bacteriostasis-induced by sulfonamides do not affect mammalian cells by this mechanism since they require preformed PGA and cannot synthesize it. Humans are, therefore, comparable to sulfonamide-insensitive bacterial that utilize preformed PGA.

- Pharmacokinetics

Except for sulfonamides especially designed for their local effects in the bowel, this class of drugs is rapidly absorbed from the gastrointestinal tract. Approximately 70% to 100% of an oral dose is absorbed, and sulfonamide can be found in the urine within 30 minutes of ingestion. Peak plasma levels are achieved in 2 to 6 hours, depending on the drug. The small intestine is the major site of absorption, but some of the drug is absorbed from the stomach. Absorption from other sites, such as the vagina, respiratory tract, or abraded skin, is variable and unreliable, but a sufficient amount may enter the body to cause toxic reactions in susceptible persons or to produce sensitization (Petri, 2001).

All sulfonamides are bound in varying degree to plasma proteins, particularly to albumin. The extent to which this occurs is determined by the hydrophobicity of a particular drug and its pK_a at physiological pH, drugs with a high pK_a exhibit a low degree of protein binding, and vice versa.

Sulfonamides are distributed throughout all tissues of the body. The diffusible fraction of sulfadiaine is uniformly distributed throughout the total body water, while sulfisoxazole is largely confined to the extracellular space. The sulfonamides readily enter pleural, peritoneal, synovial, ocular, and similar body fluids and may reach concentrations therein that are 50% to 80% of the simultaneously determined

concentration in blood. Since the protein content of such fluids usually is low, the drugs are present in the unbound form.

After systemic administration of adequate doses, sulfadiazine and sulfisoxazole attain concentrations in cerebrospinal fluid that may be effective in meningeal infections. At steady state, the concentration ranges between 10% and 80% of that in the blood. However, because of the emergence of sulfonamide-resistant microorganisms, these drugs are now used only rarely for the treatment of meningitis (Chambers ,2001).

Sulfonamides readily pass through the placenta and reach the fetal circulation. The concentrations attained in the fetal tissues are sufficient to cause both antibacterial and toxic effects.

The sulfonamides undergo metabolic alterations *in vivo*, especially in the liver. The major metabolic derivative is the N^4- acetylated sulfonamide. Acetylation, which occurs to a different extent with each agent, is disadvantageous, because the resulting products have no antibacterial activity and yet retain the toxic potentialities of the "parent substance" (Petri, 2001).

Sulfonamides are eliminated from the body partly as the unchanged drug and partly as metabolic products. The largest fraction is excreted in the urine, and the half-life of sulfonamides in the body is thus dependent on renal function. In acid urine, the older sulfonamides are insoluble and may precipitate, causing crystalline deposits that can cause urinary obstruction. Small amounts are eliminated in the feces and in bile, milk, and other secretions.

- **Pharmacological Properties of Individual Sulfonamides.**

The sulfonamides may be classified into three groups on the basis of the rapidity with which they are absorbed and excreted: (a) agents absorbed rapidly and excreted rapidly, such as sulfisoxazole and sulfadiazine; (b) agents absorbed very poorly when administered orally and hence active in the bowel lumen, such as sulfasalazine; (c) sulfonamides employed mainly for topical use, such as sulfadiazine; and (d) long-acting sulfonamides, such as sulfadoxine, which are absorbed rapidly but excreted slowly.

a. Rapidly Absorbed and Rapidly Eliminated Sulfonamides.

Sulfisoxazole. Early studies of sulfisoxazole (GANTRISIN, others) established that it was a rapidly absorbed and rapidly excreted sulfonamide with excellent antibacterial activity. Since its high solubility eliminates much of the renal toxicity inherent in the use of older sulfonamides, it has essentially replaced the less soluble agents (Petri,2006).

Sulfisoxazole is bound extensively to plasma proteins. Following an oral dose of 2 to 4g, peak concentrations in plasma of 110 to 250 µg/ml are found in 2 to 4 hours. From 28% to 35% of sulfisoxazole in the blood and about 30% in the urine is in the acetylated form. Approximately 95% of a single dose is excreted by the kidney in 24 hours. Concentrations of the drug in urine thus greatly exceed those in blood and may be bactericidal. The cerebrospinal fluid concentration averages about a third of that in the blood(Petri,2006)..

The recommended daily oral dosage of sulfisoxazole for children is 150 mg/kg of body weight: one half of this is given initially, followed by one-fourth or one-sixth of the daily dose every 6 or 4 hours (not to exceed 6g in 24 hours). The oral dosage for adults is 2 to 4 g initially, followed by 4 to 8 g daily, in four to six divided doses.

Sulfisoxazole also is marketed in a fixed dose combination with phenazopyridine (sulfisoxazole, 500 mg; phenazopyridine, 50 mg; AZO GANTRISN, others) as a urinary tract antiseptic and analgesic. The urine becomes orange-red soon after ingestion of this mixture because of the presence of phenazopridine, an orange-red dye. Sulfisoxazole acetyl also is marketed in combination with erythromycin ethylsuccinate as PEDIAZOLE OF ERYZOLE for use in children with otitis media.

Less than 0.1% of patients receiving sulfisoxazole suffer serious toxic reaction. The untoward effects produced by this agent are similar to those that follow the administration of other sulfonamides; Sulfisoxazole only infrequently produces hematuria or crystalluria (0.2% to 0.3%) because of its relatively high solubility in the urine as compared to sulfadiazine. Despite this, patients taking this drug should ingest an adequate quality of water. Sulfisoxazole and all sulfonamides that are absorbed must be used with caution in patients with impaired renal function. Like all other sulfoxamides, sulfisoxazole may produce hypersensitivity reactions, some of which are potentially lethal.Generally, the rapidly absorbed and rapidly excreted sulfonamides are preferred over other sulfonamides by most clinicians (Griffin et al., 1999).

■ **Sulfamethoxazole**

Sulfamethoxazole (GANTANOL, others) is a close congener of sulfisoxazole, but its rate of enteric absorption and urinary excretion are lower. It is administered orally and employed for both systemic and urinary tract infections. Precautions must be taken to avoid sulfamethoxazole crystalluria because of the high percentage of the acetylated, relatively insoluble form of the drug in the urine. The dosage of sulfamethoxazole for children is 50 to 60 mg/kg initially, followed by 25 to 30 mg/kg morning and evening

thereafter. The dosage for adult with mild infections is 2g , followed by 1 g every 12 hours. The clinical uses of Sulfamethoxazole are the same as those for sulfisoxazole. It also is marketed in fixed – dose combinations with phenazopyridine (AZO GANTANOL) as a urinary antiseptic and analgesic, and with trimethoprim (Petri ,2001).

■ **Sulfadiazine**

Sulfadiazine given orally is rapidly absorbed from the gastrointestinal tract, and peak blood concentrations are reached within 3 to 6 hours after a single dose. Following an oral dose of 3g, peak concentration in plasma are 50 mg/ml. About 55% of drug is bound to plasma protein at a concentration of 100 mg/ml when plasma protein levels are normal. Therapeutic concentrations are attained in cerebrospinal fluid within 4 hours after a single oral dose of 60 mg/kg (Petri,2006)

Sulfadiazine is excreted quite readily by the kidney in both the free and the acetylated form, rapidly at first and then more slowly over a period of 2 to 3 days. It can be detected in the urine within 30 minutes after oral ingestion. About 15% to 40% of the excreted sulfadiazine is in the acetylated form.

b. Poorly Absorbed Sulfonamides

Sulfasalazine is very poorly absorbed from the gastrointestinal tract. It is used in the therapy of ulcerative colitis and regional enteritis though relapses occur in one third of patients. It has also been used for some cases of regional enteritis and granulomatous colitis (Singleton, 1977; Summers *et al.*, 1979)

c. Sulfonamides for Tropical Use

■ Sulfacetamide is the N^1-acetyl-substituted derivative of sulfanilamide. Very high aqueous concentrations are not irritating to the eye and are effective against susceptible microorganisms. The drug penetrates into ocular fluids and tissues in high concentration. Although sensitivity is rare, the drug should be avoided in known cases of hypersensitivity.

■ Silver Sulfadiazine inhibits the growth *in vitro* of pathogenic bacteria and fungi, including some species resistant to sulfonamides (Rosenkranz and Rosenkrauz, 1972). The compound is used topically to reduce microbial colonization and incidence of infection of wounds from burns. It is not used to treat established infection, silver is released slowly from the preparation in concentrations that are selectively toxic to the microorganisms. However, bacterial may develop resistance to silver sulfadiazine (Wenzel *et al.*, 1976). While little silver is absorbed, the plasma concentration of sulfadiazine may approach therapeutic levels if a large surface area is involved. Adverse effects include burn, rash and itching (Ballin, 1974).

■ **Mafenide**

It has been used in burns, applied topically to prevent colonization by a large variety of gram negative and gram – positive bacteria. Superinfection with candida occasionally occurs. Mafenide is rapidly absorbed systemically and converted to para – carboxybenzenesulfonamide with peak plasma concentrations in 2 to 4 hours (Harrison *et. al.*, 1972). Adverse events include pain, loss of fluids at the site of application and allergic reactions. Allergic reactions also occur. The drug and its primary metabolite inhibit carbonic analydrase. The urine becomes alkaline, and a metabolite acidosis may occur (White and Asch, 1971).

Compensatory tachypnea and hyperventilation with respiratory alkalosis are also observed.

- **Long Acting Sulfonamides which are Rapidly Absorbed but Slowly Excreted**

■ Sulfadoxine

Sulfadoxine inhibits the activity of dihydropteroate synthase. It is active against the asexual erythrocytic stages of *Plasmodium falciparum* and may also be effective against strains of *P. falciparum* resistant to chloroquine. Strains of *P. falciparum* with decreased susceptibility to sulfadoxine can be selected *in vitro* or *in vivo*. *P. falciparum* malaria that is clinically resistant occurs frequently in parts of Southeast Asia and South America, and is also prevalent in Africa. Therefore, sulfadoxine should be used with caution in these areas. Likewise, sulfadoxine may not be effective for treatment of recrudescent malaria that develops after prior therapy (or prophylaxis) with sulfadoxine. After administration of 1 tablet of the combination sulfadoxine- pyrimethamine, peak plasma levels for sulfadoxine (approximately 60 mg/L) are reached after about 4 hours. The volume of distribution for sulfadoxine is 0.14 L/kg.Patients taking 1 tablet a week of sulfadoxine-pyrimethamine (recommended adult dose for malaria prophylaxis) can be expected to have mean steady state plasma concentrations of about 98 mg/L for sulfadoxine after about seven weeks. Plasma protein binding is about 90%. Sulfadoxine crosses the placental barrier and passes into breast milk. About 5% of sulfadoxine appears in the plasma as acetylated metabolite, about 2 to 3% as the glucuronide. A relatively long elimination half-life is characteristic. The mean value is about 200 hours and elimination is mainly via the kidneys. In patients with malaria, single pharmacokinetic parameters

may differ from those in healthy subjects, depending on the population concerned. In patients with renal insufficiency, delayed elimination of the components of the drug combination must be anticipated. Though many patients and prescribers still use sulfadoxine as single therapy drug, the ideal practice is that it should be given in combination with artemisinin especially in regions where resistance is common for treatment of acute malaria. Malaria prophylaxis with sulfadoxine-pyrimethamine is not routinely recommended and should only be considered for travelers to areas where chloroquine-resistant *P. falciparum* malaria is endemic and sensitive to this drug, and when alternative drugs are not available or are contraindicated. However, strains of *P. falciparum* may be encountered which are resistant. Repeated prophylactic (prolonged) use of sulfadoxine is contraindicated in patients with renal or hepatic failure or with blood dyscrasias, also in patients with hypersensitivity to sulfonamides and those with documented megaloblastic anemia due to folate deficiency. Prophylactic use in pregnancy at term and during the nursing period is contraindicated. Infants less than 2 months of age should not be given this drug (FMOH, 2008).

- **Untoward Effects**

The margin between toxicity for bacteria and humans as regards antifolate action may be narrow when patient's cells already have folate deficiency. In such cases trimethoprim-sulfamethoxazole may cause or precipitate megaloblastosis, leukopenia, or thrombocytopenia. About 75% of the untoward effects involve the skin, trimethoprim-sulfamethozole has been reported to cause up to three times as may dermatological reactions as does sulfisoxazole when given alone (Arndt and Jick, 1976) Exfoliative dermatitis, Stevens – Johnsons syndrome, and toxic epidermal necrolysis (Lyell's syndrome) are rare, occurring primarily in older individuals.

Nausea and vomiting constitute the bulk of gastro-intestinal reactions, diarrhea is rare. Glossitis and stomatitis are relatively common. Mild and transient jaundice has been noted. Central Nervous system reactions include headache, depression and hallucination. Hemotological reactions also include anaemia, coagulation disorders, gramulocytopenia, agranulocytosis, purpura, Henoch – Schonlein purpura and sulfhemoglobinemia. Previous or simultaneous administration of diuretics with trimethoprim – sulfamethoxazole may carry an increased risk of thrombocytopenia especially in elderly patients with heart failure; death may occur,permanent renal function impairment may follow the use of trimethoprim – sulfamethoxazole in patients. The use of trimethoprim – sulfamethoxazole in patients with renal disease (Kalowski *et al.*, 1973) and a reversible decrease in creatinine clearance has been noted in patients with normal renal function (Shouval *et al.*, 1978)

2.4.5.1.5 ARTEMISININ AND ITS DERIVATIVES

- **History**

Artemisia, Qinghaosu and Wormwood are derived from the herb Artemisia annua, in China known as ‘qinghao’ (Chinese for “from green herb”), which is a member of the ‘Asteraceae’ family. Qinqhao, notwithstanding a bitter taste also known as ‘sweet Annie’ or ‘sweet wormwood’, is a fragrant annual herb, which now grows wild in a number of countries, including Australia, Argentina, Bulgaria, France, Hungary, Italy, Spain and the United States. It is native to Asia and has been used as antiparasitic therapy for malaria in Traditional Chinese Medicine (TCM) for more than 1,000 years (WHO, 2008).The earliest reference to the medicinal use of artemisia annua in ‘The Fifty Two Prescriptions’ which was uncovered in ancient Chinese burial tombs during an archaeological dig in the 1970s, dates back more than 2,000 years. There are more than 300 species that comprise the genus Artemisia, many of which are sources of

herbal medicines, spices or essential oils. Chinese chemists isolated the primary active ingredient in artemisia annua from the leafy portion of the plant in 1972 and called the crystalline compound, 'qinghaosu' - or 'artemisinin' in the West. Artemisinin is a sesquiterpene lactone that bears a peroxide group and unlike most other antimalarials, lacks a nitrogen-containing heterocyclic ring system. It is poorly soluble in water and decomposes in other polar solvents, probably by opening of the lactone ring. It is also poorly soluble in most none polar solvents and can thus only be administered orally. It shows a remarkable thermal stability. The peroxide moiety of artemisinin has appeared to be indispensable for chemotherapeutic activity (Meshnick *et al.*,1993). *In vivo* artemisinin is metabolised to dihydroartemisinin in which the integrity of the peroxide group is retained.

- **Antischizontocidal action**

Artemisinin derivatives act as blood schizontocidal agents effectively inhibiting the late stage ring parasites and trophozoites. They kill the Plasmodium parasites more quickly than any other antimalarial agent and are toxic to the parasite at very low concentrations. Thus far, no *in-vivo* resistance has been described, making them also effective in the treatment of multiresistant malaria (WHO, 2008).

- **Mechanism of action**

Artemisinin reacts in the parasite with iron (Meshnick *et al.*, 1993; Meshnick, 1994; Meshnick, 2002). The haemoglobin within the infected erythrocyte is digested by the parasite; haem is released and neutralized by polymerisation into haemazoin with a high Fe2+ content. When artemisinin comes into contact with high iron concentrations, a chemical reaction is produced which creates free radicals that attack

cell membranes, breaking them apart and killing the single-cell parasite. The 'explosive' nature of this mechanism of action of artemisinin and its derivatives is of interest and gave rise to the hope that it could prevent any rapid occurrence of resistance.

- **Pharmacokinetics**

In patients with severe malaria, oral treatment is often impossible and thus parenteral formulations were required. Therefore water soluble artesunate, the hemi-succinate of dihydroartemisinin and the oil soluble arteether have been developed for intravenous and intramuscular administration respectively (White, 1994). Development of oil soluble artemether was promoted by WHO and the water-soluble artelinic acid by the Walter Reed Army Institute of Research. Oral formulations of artemisinin and its derivatives are absorbed rapidly but incompletely with considerable interindividual variability. Peak plasma concentrations are reached in 1-2 hours and most compounds have a short elimination half-life of 1-3 hours after oral intake. Artesunate acts like a prodrug with fast transformation into dihydroartemisinin and it has an elimination half-life of less than half an hour. Intramuscular and rectal dosing exhibit slower and more variable absorption and elimination. For arteether an elimination half-life of 23 hours has been reported in dogs and also in healthy subjects after a single intramuscular dose (Kager *et al.*, 1994). It is thus predictable, and it has also been shown that arteether will cumulate after multiple doses. A striking pharmacokinetic phenomenon in multiple dose studies of some artemisinin analogs is a time dependent decrease in plasma concentrations, probably caused by autoinduction. Related to the short elimination half-lifes of most artemisinin drugs is the occurrence of recrudescences when they are given in short course monotherapy regimens. Therefore

combination with a longer acting antimalarial drug is usually recommended. Characteristic for the artemisinin drugs is their swift onset of action with clearance of parasites from the blood within 48 hours in most cases. A meta-analysis showed a survival-benefit with artemisinin drugs compared to quinine in the treatment of severe (complicated or cerebral) malaria. There is no evidence from randomised trials that one artemisinin derivative is better than the others. In areas where there is mefloquine resistance, combination therapy with an artemisinin derivative appears to improve sustained parasite clearance compared with either drug alone. It can be concluded that artemisinin and its derivatives are a major breakthrough in the fight against a very prevalent and deadly disease (Hien and White, 1993).

- **Adverse Drug Reactions**

These drugs have surprisingly few adverse effects (Price *et al.*, 1998). Regimens of Artemisinin derivatives given to patients with acute falciparum malaria as single agents were associated with the following mild side effects; acute nausea (16%), vomiting (11%), anorexia (34%), and dizziness (15%). Nevertheless, repeatedly, concern has been expressed centering on possible neurotoxicity. (Brewer *et al.*, 1994). In all experimental mammals tested (rats, dogs, primates), intramuscular injections of the oil-soluble antimalarial artemisinin derivatives artemether and arteether have produced an unusual pattern of selective damage to brain stem centers predominantly involved in auditory processing and vestibular reflexes. Neurological findings included gait disturbance, loss of spinal and pain response reflexes, and prominent loss of brain stem and eye reflexes. Artemether dose-dependent neuropathologic damage to the brain stem was shown in the mouse (Nontprasert *et al.*, 2002). The

neurons in the lower brain stem trapezoid nucleus, the gigantocellular reticular nucleus, and the inferior cerebellar peduncle were the most sensitive to the toxic effects of artemether. All mice with neuropathologic changes also showed behavioural changes. Most importantly, *in vitro* it was shown that Artemisinin induces oxidative stress in cultured neurones, as concluded from an increase of reactive oxygen species and extensive lipid peroxidation (Schmuck *et al.*, 2002). Especially in brain stem cultures a markedly deficient induction of antioxidant enzymes was observed. Malaria itself can cause cerebral symptoms. Cerebral malaria induces changes in mental status and coma and is the main cause of death in *Plasmodium falciparum* malaria. The histopathological hallmark of this encephalopathy is the sequestration of red blood cells in cerebral capillaries and venules and it has been shown that reduced red cell deformability contributes to the derangement of the microcirculation (Dondorp *et al.*, 2002, Chotivanich *et al.*, 2000). High concentrations of artemisinin cause oxidation of red blood cell membrane proteins and then also decrease red cell deformability, especially in the presence of exogenous haem (Meshnick, 1994; Sibmooh *et al.*, 2003). It can therefore not be excluded that these agents may affect plasma membrane function in infected erythrocytes. It can be concluded that at least one species of artemisia can have serious adverse neurological reactions in humans.

Furthermore, the mechanism of action of artemisinin through the formation of free radicals is potentially toxic in nature. In experimental animals neurological damage could be induced with several artemisinin derivatives and *in vitro* artemisinin, through an increase of reactive oxygen species, induces oxidative stress in cultured neurones. Finally, it is not excluded that artemisinin derivatives may influence the deformability

of red blood cells and that therefore their possible neurotoxicity could masquerade as cerebral malaria.In humans treated with the artemisinin drugs adverse effects that could directly be ascribed to neurotoxicity have yet to be demonstrated. The recommended dosages for the treatment of malaria however seem to be too low to pose much toxicological risk.

2.4.6 THERAPEUTICS

The effectiveness of early diagnosis and prompt treatment as the principal technical components of the global strategy to control malaria is highly dependent on the efficacy, safety, availability, affordability and acceptability of antimalarial drugs. The effective antimalarial therapy not only reduces the mortality and morbidity of malaria, but also reduces the risk of resistance to antimalarial drugs (WHO, 2008).

Therefore, antimalaria chemotherapy is the keystone of malaria control efforts. On the other hand, not many new drugs have been developed to tackle malaria. Of the 1223 new drugs registered between 1975 and 1996, only 3 were antimalarials. For this reason, there is the need for a rational antimalaria treatment policy.

Treatment of malaria can either be for prophylactic or curative purposes. Prophylaxis includes the use of drugs to eradicate the primary tissue forms in the liver destined to initiate an infection and also drug treatment to eradicate latent tissue forms of *P. vivax* and *P. ovale,* which would otherwise have remained after the primary tissue forms are released at the beginning of the infection. Proguanil, pyrimethamine, chloroquine, sulphadoxine /pryimethmine and mefloquine have been used for prophylaxis over the years. However, optimal prophylactic effects can be got presently in view of

resistance formation when atovaquine is combined with proguanil. Cure of infection by latent tissue forms otherwise known as prevention of relapse is best achieved with the use of primaquine. For curative treatment, drugs which interrupt erythrocytic schizogony and therefore terminate clinical attacks (or achieve clinical cure) are used. These act on asexual erythrocytes. There are the rapidly acting, such as chloroquine, amodiaquine, quinine, mefloquine, artemisinin, and atovaquone. Also, the slower acting, less effective drugs which include sulphadoxine /pyrimethamine and doxycycline can be used for clinical cure. The standard therapy recommended for curative purposes, in endemic zones presently in view of resistance (Rolfe, 1998), is a combination of artemisinin and another drug such as amodiaquine, lumifantrine, mefloquine or sulphadoxine /pyrimethamine combination (WHO, 2007; WHO, 2005; White *et al.*, 1999). This is known as Artemisinin Based Combination Therapies (ACTs). Chloroquine is given in a dose of 25mg/kg body weight usually over a 3-day period. It is usually given through the intramuscular, oral, or subcutaneous route.

Amodiaquine can also be given as 25mg/kg body weight orally over a 3 day period, though it is usually prescribed in a single dose fashion of 750mg *statum* for adults or its weight equivalent for children. This single dose may be repeated once or twice according to the physician's judgment of the level of the patient's immunity or the severity of the infection. Used this way, an adult patient thus takes a total of 3, 6, or 9 tablets.

Quinine is normally given orally or through the intravenous route for patients in whom the oral route may not be advisable. However, when the patient can tolerate the oral route or has recovered from the severe state, an oral schedule should be initiated

promptly. The dose is 10mg/kg (maximum 650mg of the salt) dissolved in 300ml of normal saline infused over 1 to 2 hours, followed by a continuous infusion of 0.02 mg of salt per kg per minute, until an oral therapy is feasible. Monitor BP for hypertension, ECG for widening of QRS complex and lengthening of QT interval continuously, total blood glucose (for hypoglycaemia) periodically. The oral dose, quinine sulphate is given as 650mg salt every 8 hours. The paediatric dose is 10mg of salt /kg every 8 hours. The dose should be decreased by 30% to 50% after 48 hours if there is no clinical improvement. The standard duration of therapy is 7 days (FMOH,2008).

Mefloquine mono therapy is not as popular as it was previously. It is best utilized now for a patient in whom there is no contraindication especially of the central nervous system disease and who suffer from *P. falciparium* resistant to other drugs. It is given in combination with artemisinine for 3 to 5 days at a dose of 25mg/kg body weight once or split. Adults take 1250 mg stat and 750mg 12 hours later. Artemisinin, usually given orally is prescribed at a dose of 4mg/kg body weight on day 1, and 2mg/kg body weight on the subsequent days. It is advised that it be taken for at least 5 to 7 days to prevent resistance formation. Atovaquone 1000mg is usually combined with proguanil 400mg both given daily for 3 days. Sulphadoxine /pyrimethamine combination is not as effective against *P. falciparium* as it was previously(Naing *et. al.*, 1988; Keschamrus *et al.*, 1988).It is better prescribed in combination with other antimalarial drugs, at an adult dose of 1500mg/75mg of sulphadoxine and pyrimethamine, respectively, for greater effects (Naing *et. al.*, 1988).The dose for children is as follows**:** less than 1 year of age: 0.25 of the standard tablet, 1 to 3 years

(weighing 5 to 10 kg): 0.5 of the tablet, 4 to 8 years (11 to 20 kg): 1 tablet, 9 to 14 years (31 to 45 kg): 2 tablets, 15 years and older or over 45 kg: 3 tablets.

Doxicycline is prescribed at a dose of 100mg 8 hourly for 7 days. For children over 8 years, 2mg /kg body weight increased up to adult dose. Tetracycline is prescribed at a dose of 250mg 6 hourly over a period of 7 days. For children over 8 years, 5 mg /kg body weight. Tetracyclines are contraindicated in children less than 8 years of age, pregnant women and patients hypersensitive to the drug. Primaquine therapy is commenced after the acute attack (about day 4) at a dose of 26.3 mg (15 mg base) daily for 14 days. Paediatric dose is 0.53 mg/kg (0.3 mg base per kg body weight) daily for 14 days (Rathbun, 2000).

2.4.7 PREVENTION AND CONTROL

The WHO in 2005 defined key elements of malaria control (WHO, 2005). These are as follows:

1. Early diagnosis and prompt treatment
2 Prevention, including vector control and insecticide treated net (Rozendaal, 1989).
3 Early detection, containment and prevention of epidemics.
4 Strengthening national capacity for research and monitoring.

Many different approaches have been taken to prevent the cycle of disease and mortality from malaria. Some of these approaches are:

Modifying the environment, by eliminating stagnant water where they can breed, as well as spraying DDT and other insecticides indoors. This has worked in specific areas including Southern Africa (Botswana). In other countries such as Brazil and Sri Lanka, it has been used to significantly reduce malaria transmission or to eliminate malaria as was the case in the United States. This was the basis of targeted malaria control efforts in the past. However, these resulted from the eradication era with a widespread and costly effort. Resistance to DDT as well as technical, economic and structural problems has limited the achievements of the residual spraying programme of the post-war eradication era in much of Africa, where transmission is from hyper endemic to holo-endemic and effective control programmes are rare.

Window screens have been used to limit the chances for human exposure to the infected mosquito. Prevention of the disease has been achieved through prophylactic use of antimalarial drugs. This has however proved the ability of the parasites to develop resistance to the more commonly used drugs(Iyer et al.,2001).

It is anticipated that it will be possible in the future to prevent human diseases through the use of vaccines. This is the goal of the Malaria Vaccine Institute (MVI).

It has now been convincingly demonstrated that the use of bed nets and curtains treated with insecticide substantially reduces mortality and morbidity from malaria, at least in the short term. Insecticide treated bed nets now provide residents of malaria endemic areas with an effective means of protecting themselves against malaria (Rozendaal, 1989; Takken, 2002). The public health priority now is to find ways of

making nets widely available, educating people about their use, and finding ways of making them more affordable.

2.4.8 MALARIA VACCINE RESEARCH

The need for a vaccine against this disease is intense, as mortality and morbidity associated with malaria are increasing. Despite the disease's long history and the existence of a multitude of treatments, around 2 to 3 million lives are still lost every year because of the disease with approximately 90% of these in sub-Saharan Africa. An estimated 2 billion people live in areas where malaria is transmitted, with between 300 and 500 million new infections occurring in this group every year. Individuals who have not developed immunity to the infection are most at risk from the disease. Infection with *P. falciparium* causes much of the mortality, which preferentially affects children less than 5 years of age, travellers, pregnant women and non - immune individuals (Tracy and Webster, 2001).

The global burden of malaria is increasing due to the development of drug resistant parasites and insecticide resistant mosquitoes; this is illustrated by re-emergence of the disease in areas that were previously safe. The world is becoming less well equipped to deal with malaria and the present morbidity and mortality trends are set to continue unless new methods of control are developed. The implications of an increasing burden on the public health infrastructure and economic stability of the countries most badly affected is cause for concern, therefore making alternatives to the currently available treatment options and prevention strategies research priorities.

Vaccines are one of the most effective modes of treatment available for various diseases. They are cost-effective in most instances and easily administered. They have

historically presented a significant reduction in the spread and burden of infectious diseases. Yet no effective vaccine for malaria has so far been developed. Despite this, the researchers remain hopeful. Optimism is justified for several reasons. The first of these being that individuals who are frequently exposed to the parasite for long periods of time gain an acquired immunity against developing any clinical manifestation, even when the parasitic infection is evident by blood film analysis. Also, a new approach uses spores of the fungus *Beauveria bassiana*, sprayed on walls and bed nets, to kill mosquitoes (Scholte *et al.*, 2005). While some mosquitoes have developed resistance to chemicals, they have not been found to develop a resistance to fungal infections.

2.4.9 HERBS USED IN TREATING MALARIA

The number of plants used to treat malaria or fever in traditional medicine appears almost unlimited. Some species of plants are used as anti-malarials or antipyretics in all three tropical continents (Willcox and Bodeker, 2004). This world-wide experience showing more or less efficacy of various different plant species to clear parasites and fever suggests that the chemical structures of effective plant components may differ considerably. Recently it was found that extracts from various plants used in traditional medicine such as Uncaria tomentosa, Hypericum perforatum and also Camellia sinensis significantly suppressed the production of interferon-g and of neopterin in stimulated peripheral blood mononuclear cells (Reibnegger *et al.*, 1984; Brown *et. al.*, 1990). From the data it appeared that especially antioxidant plant compounds were responsible for the immunosuppressive effect.

Malaria and other parasitic infections are associated with, significantly increased neopterin concentrations in blood and urine (Winkler *et. al.*, 2004; Winkler *et. al.*, 2005), which indicates immune activation and production of Th1-type cytokine interferon-g. Immune activation certainly is involved in the development of at least part of typical symptoms in patients including fever. Thus, especially the antipyretic effect of various plants used to treat malaria appears to be related to the antioxidant content of plants and plant extracts. Upon further characterization of the plant compounds involved in the anti-malarial and antipyretic activity, new immuno-modulators and new therapeutic applications may result.

2.5.0 INFLUENCE OF NUTRITIONAL FACTORS ON PHARMACOKINETIC PARAMETERS

Pharmacokinetic parameters which include absorption, distribution, metabolism and excretion of drugs may be modified by the patient's nutrition (Wilkinson,2001).The consequence may be an increased drug action or treatment failure (Wilkinson,2001). When patients with HIV and malaria experince such modification of drug action, it may subsequently alter the action of any antimalaria drug administered and eventually their malaria status.

Three important factors involved in drug absorption are the pH of the gastrointestinal tract, the fat content of the tract, and transit time of the drugs. Some drugs are better absorbed in the stomach which contains gastric juice, an acidic medium, while others are preferentially absorbed in the alkaline regions of the small intestine (Wilkinson,2001). When the stomach is relatively empty, the pH tends towards acidic level hence, an acidic drug will be better absorbed there.

The prensence of much food in the stomach may on the contrary slow down absorption of an acidic drug. The absorption of drugs such as atovaqoune may

increase remarkably in the presence of fatty meals. Inadequate nutrition with consequent malnutrition may present with a reduction of plasma protein. Drugs such as Mefloquine bind to plasma protein on a massive scale (Tracy and Webster, 2001). When there is a significant reduction in the concentration of plasma protein in the human body, the concentration of the unbound forms of drugs which would otherwise have bound to plasma protein is increased. This unbound fraction will exert more pharmacological actions and can lead to drug toxicities.

A malnourished patient may have low body fat content which will determine the eventual amount of drugs which will partition into the body fat. This is however of importance for drugs which have high degree of fat solubility (Rang *et al.*,2008). Apart from the role of body protein content in the determination of plasma protein concentration and distribution of drugs, through binding to plasma protein, there is also a role played by plasma protein in drug in drug elimination. This is because a relatively high proportion of unbound drug in cases of hypoproteinaemia can be cleared through the glomeruli rather quickly (Rang *et al.*, 2008).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.0 Pharmacoepidemiological Studies

3.1 Knowledge, Treatment and Preventive Practices of Malaria.

3.1.1 The Study Area:

This study was carried out in the Lagos University Teaching Hospital (LUTH), Idiaraba, Lagos.

The Lagos University Teaching Hospital is a tertiary health training hospital, one of the 2 located in Lagos State, South West Nigeria. It is in the Lagos Mainland. The hospital has admission facility for 761 patients and runs outpatient clinic units. There is a full complement of departments reflecting the various medical specialities. The Department of Hematology and Blood Transfusion hosts the HIV/AIDS Clinic.

3.1.2 Study Design:

A structured questionnaire adopted from a previous study (Hamel *et al*., 2001) was drawn up for the study and administered by oral interview method (Appendix A). The questionnaire was divided into 3 sections. The first section contained the identification number of the patient, date and demographic characteristics including age, sex, educational qualification, employment status, occupation, marital status, spouse's occupation and employment status and family's income. The other section contained information on patient's knowledge of malaria, treatment seeking practices when the patient feels afflicted with malaria and the preventive measures the patient takes against malaria fever.

The study population consisted of adult patients attending the HIV/AIDS clinic, in the LUTH. Those selected for the study were consecutive patients who agreed to be

enrolled after explanation of the purpose of the study and what it entailed. Out of 741 patients met, 469 accepted to participate in the study.

3.2 The Prevalence of Malaria Parasitaemia among HIV Infected Patients without features of Clinical Malaria.

3.2.1 Ethical clearance was obtained from the Ethics Committee, Lagos University Teaching Hospital (Appendix B).

3.2.2 Written informed consent was obtained from the patients and subjects. (Appendix C).

3.2.3 Selection of Subjects.

One hundred consecutive patients with HIV/AIDS, but without clinical features suggestive of malaria, were recruited into the study. Inclusion criteria included domicile in Nigeria in the last 1 month period prior to the study. Exclusion criteria included fever, and use of drugs with antimalaria action in the last one month. Healthy, non-HIV infected adult subjects in the general population (n =100) domiciled in Nigeria in the last 1 month were recruited as controls.

3.3. Relationship between Clinical Malaria and Parasitaemia among HIV/AIDS Patients.

3.3.1 Selection of Subjects.

One hundred consecutive adult patients with HIV infection and diagnosed clinically as cases of malaria fever at the HIV Clinic, LUTH were recruited into the study. Inclusion criteria included fever of at least 37.5^{o}C, domicile in Nigeria for at least 1 month period prior to the research. Informed consent form was filled by the patients.

HIV negative patients with clinical features suggestive of malaria (n =100) were selected as controls.

3.3.2. Determination of Malaria Parasite Count

3.3.2.1 Collection of Human Blood Specimen

The skin over the antecubital fossa where the brachial vein was to be bled was cleaned with 70% ethanol for the purpose of antisepsis. A tourniquet was applied in the mid upper arm to dam the blood flow in the veins, 2 mℓ of the patient's blood sample was obtained, part of this was applied on a slide for the purpose of malaria parasite microscopy.

3.3.2.2. Barrier Measures

Standard protocols to prevent infection of researcher or patients in the course of this study were observed. These included the use of gloves while handling any potentially infected material, proper handling of sharp objects up to the level of the disposal of these objects.

3.3.2.3. Preparation of the Thick and Thin Blood Films.

A thin and a thick film were made on the same slide. The thin film was used as a label and for species confirmation, while the thick film was used for detection of parasites and examination.

3.3.2.4. Thin Film

A single small drop of blood was applied on the middle of the slide. The slide with the blood drops was placed on a flat, firm surface. The small drop of blood was touched with another clean slide to be used as a "spreader" placed at an angle of 45^0 to the horizontal slide. The drop of blood was allowed to run along the edge of the spreader. With a firm push the spreader was run along the horizontal slide while maintaining an even contact between the surfaces all the time the blood was being spread. The spread stopped before the edge of the horizontal slide (WHO, 2009).

3.3.2.5. Thick Film

Three larger drops of blood were applied onto the same slide about 1 cm from the drop intended for the thin film. Using the corner of the spreader, the larger drops of blood was joined quickly and spread in a circular form to make an even thick film with 3 – 6 movements. The thick film was allowed to dry in a flat, level position protected from flies, dust and extreme heat.

3.3.2.6. Staining the Blood Films with Giemsa Stain.

The blood film was allowed to dry overnight. The thin film was fixed by adding 3 drops of methanol for a few seconds. Caution was applied to prevent fixation of the thick film. The slides were placed back to back in a staining dish. Enough quantity of 3% Giemsa solution prepared in buffered distilled water, pH 7.2, was poured in gently to fill the dish until the slides were totally covered. The smears were allowed to stain for 30-45 minutes away from the sunlight. The whole dish was immersed in a vessel filled with clean water. The remaining stain was poured out gently, and the dish was rinsed again in clean water for a few seconds and the water poured off. The slides was

removed one by one and placed in a slide rack to drain and dry, film side downwards, making sure that the film did not touch the slide rack (WHO, 2009).

3.3.2.7. Examination of the Thick Films.

The malaria parasites were viewed with the oil immersion objective lens. At least 100 fields were examined.

3.3.2.8. Examination of the Thin Films.

The x10 objective lens was focused on the thin terminal end of the film where the red blood cells were in one layer. The immersion oil was then applied on the slide and the lens switched to the oil immersion objective. Two hundred fields were examined.

3.3.2.9. Establishing a Parasite Count.

Development of parasitaemia was monitored by microscopic examination following the methodology of Fern, McNurtan and Garlick (Shida *et al.*, 1989). This was done with the thick film. The number of parasites per micro litre of blood in the thick film was counted in relation to a standard number of leukocytes (8000).Two tally counters were used, one to count the parasites and the other to count the leukocytes.

Step 1(a) If, after 200 leukocytes had been counted, 10 or more parasites were identified and counted; the result was recorded in terms of the number of parasites per 200 leukocytes.

(b) If, after 200 leukocytes had been counted, 9 or fewer parasites were counted, the counting was continued until 500 leukocytes were counted, then the number of parasites were recorded per leukocytes.

Step 2. In each case the number of parasites relative to the leukocyte count was converted to parasite per micro litre of blood by the mathematical formula:

Number of parasites x 8000 / Number of leukocytes = parasites per micro litre.

3.4 Some Heamatological Patterns of HIV Patients and Relationship of Hemoglobin Concentration, CD_4 Count, Blood Group, Genotype, and Viral Load to Malaria Parasitaemia.

3.4.1. Laboratory Investigation

Five ml venous blood sample were taken and used for the various assays.

3.4.1.1. Determination of Malaria Parasites

Determination of malaria parasites in peripheral blood was ascertained by thick and thin blood smears stained with Giemsa. Parasite density per microlitre was estimated.

3.4.1.2. Hemoglobin Concentration (Cyanmethemoglobin Method)

The cyanmethemoglobin method (Drabkin and Austin, 1935) was used, 5 ml of Drakins reagent, were added to 0.02ml of whole blood previously collected in an EDTA bottle. The 2 substances were mixed and allowed to stand for 10 minutes at room temperature. The Optical density was read in a spectophotometer at a wavelength of Hg 546 nm; 640nm. The hemoglobin concentration was calculated comparing the Optical Density of the patient's specimen with that of a standard using the formula below:

Optical Density of patient's specimen x 20 = g of hemoglobin/dl Optical Density of standard.

3.4.1.3. CD_4 Count

The flow cytometer method was used (Alexander *et al.*,1994) A whole blood volume of 20 µl previously collected in an EDTA bottle was added to a Partec test tube. Subsequently, 20 µl of the reagent, CD_4 mAb PE(MEM – 241, PE – conjugated monoclonal antibody to human CD_4) was added , followed by 800 µl of no lyse buffer. The contents of the Partec test tube were mixed by shaking it and CD_4 – PE fluorescence analysis was done on a Partec Flow Cytometer with an excitation light source of 488nm.The CD_4 count analysis was obtained as CD_4^+ T – cells per µl whole blood (Alexander *et al.*,1994 **;** Nicholson *et al.*,1996).

3.4.1. 4. Blood and Rhesus Grouping (Tile Method)

The tile method of blood grouping was used (Landsteiner and Levine 1927). An opal glass tile etched into 1-inch squares, to prevent running together of the cell reagent suspensions was used for the two tests. One volume of 20 percent patients' red cells was added to 1 volume each of anti-A and anti-B and mixed with an applicator stick to a diameter of approximately 20 mm. The presence of agglutination was used to determine whether the patient's blood group was A, B, O, or AB. Similarly, the patient's blood specimen was added to commercial anti-D sera and mixed together. D positive and negative cells were included as controls. The presence of agglutination was used to determine Rhesus D positive and negative patients.

3.4.1.5. Genotyping (Cellulose-Acetate Electrophoresis Method):

The Cellulose-Acetate Electrophoresis method was used (Herbert, 1993)

Electrophoretic tank, Whatman No 3 filter paper, Cellulose acetate strips. Buffer Tris (Tris – EDTA –borate) (pH): Tris-(hydroxymethyl)-amino – methane 10.2g, EDTA 3.2g, boric acid 0.6g, water to 11.

Separation of Hemoglobin Cellulose acetate strips, 140 x 120 mm, were soaked in the tris buffer and gently blotted. The strips were placed in an electrophoretic tank and secured at each end by a double layer of Whatman No. 3 filter paper. Subsequently, 2 uℓ of hemoglobin solution was applied in a 2 – cm line midway between the centre of the strip and its cathodal end. Electrophoresis was carried out for 2 hours at 5 mA at a potential of 220 V across the strip. At the end of the separation, the membrane was removed with forceps and dried in a hot – air oven at 80 - 100 ^{o}C or 10 – 30 minutes. The bands were inspected without staining (Herbert, 1993).

3.4.1.6. Determination of the Viral Load

The AMPLICOR HIV – 1 MONITOR Test, version 1.5, an *in vitro* nucleic acid amplification test for the quantification of Human Immunodeficiency Virus Type 1 RNA in human plasma was used (Randall *et al.*, 1988, Mullis *et al.*, 1987). The Polymerase Chain Reaction technology was used. The specimen was collected in an EDTA bottle. The sample was prepared using the standard procedure which involves lysing the viral particles with a chaotropic agent followed by the precipitation of RNA with alcohol in order to isolate the HIV 1 RNA. A known number of quantitation standard RNA molecules was introduced into each specimen with the lysis reagent. The HIV 1 Quantitation Standard was carried through the specimen preparation, reverse transcription, amplification, and detection and was used for the quantification of HIV-1 RNA in the test specimen. The amount of HIV 1 RNA in each speciemen is

calculated from the ratio of the Total HIV 1 OD to THE Total HIV-1 Quantitation Standard OD and the input number of HIV-1 Quantitation Standard RNA molecules using the following equation:

$$\frac{\text{Total HIV-1 OD}}{\text{Total QS OD}} \times \frac{\text{Input HIV-1 QS copies}}{\text{PCR}} \times \text{Sample Volume Factor} = \text{HIV-1 RNA copies /m}\ell$$

Where:

Total HIV – 1 OD = calculated Total OD for HIV 1 amplicon

Total QS OD = calculated Total OD for HIV Quantification Standard amplicon

Input HIV – 1 QS copies/PCR = the number of copies of Quantification Standard in each reaction

Sample Volume Factor = factor to convert copies/PCR to copies/mL

Where:

Sample Volume Factor for Standard specimen preparation procedure = 40

Sample Volume Factor for Ultra Sensitive specimen preparation procedure = 4

3.4.1.7. HIV Test

Detect HIV Test (version 4), a fourth generation immunoassay was used for the diagnosis of HIV-1 and HIV-2. Antigens representing immunodominant epitopes of HIV-1 gp 41 and HIV-2 gp 36 as well as antibodies against the antigen p 24 are coated onto wells of a microplate. Plasma samples were added to these wells and if p 24 antigens or antibodies specific for HIV-1 or HIV-2 were present in the sample, they formed stable complexes with the HIV antigens or antibodies. .Antigen-antibody complexes were then identified through the successive addition of Biotinylated antigen and antibodies and Horseradish peroxidase Sreptavidin conjugate

respectively. The catalytic activity of Horseradish peroxidase allows for the quantification of these antibody-antigen complexes. Peroxidase substrate solution was then added. During incubation, a blue colour developed in proportion to the amount of anti-HIV 1 / 2 antibodies and p 24 antigens bound to the complex, thus establishing their presence or absence in the sample. Wells containing samples negative for anti-HIV antibodies and p 24 antigens remained colourless. A stop solution was added to each well and the resulting yellow colour was read on a microplate reader at 450nm (Kuritzkes,2004).

3.5. Relationship between Malaria Parasitaemia and Medication used by HIV Infected Patients.

3.5.1. Selection of Subjects

One hundred consecutive adult patients with HIV infection were recruited into the study after obtaining their informed consent.

3.5.2. Survey of Drug Use

A structured questionnaire was used by the interview method to obtain information on the use of various medications by the patients (Appendix D). The case notes of the patients were also used to obtain details of drug prescriptions given to the patients at the HIV clinic in LUTH. The questionnaire was divided into 2 main sections. The first section contained the identification number of the patient, date and demographic characteristics including age, sex, educational qualification, employement status marital status, occupation, spouse's occupation and employment status. The family's monthly income was also ascertained. The second section contained information

about the names, dosages of the various drugs utilized by the patient over the last 28 days prior to presentation. Under this section the drugs were classified into those prescribed at the HIV/AIDS clinic, those prescribed at other health facilities outside LUTH and non-physician prescribed drugs, herbs or native concoctions.

3.6 *In Vivo* Studies - Evaluation of the Antiplasmodial effect of some Antiretroviral drugs on *Plasmodia berghei* infection in mice.

3.6.1 Parasite

The NK 65 strain of *Plasmodium berghei* used in this study was obtained from the Malaria Unit, Nigerian Institute of Medical Research (NIMAR), Yaba, Lagos and maintained in mice by weekly passage. Each mouse for the curative experiment was inoculated on day 0 intraperitoneally with 0.2ml of infected blood containing about 1 x 10^{7} *P. berghei* parasitized red blood cells obtained from a donor mouse having about 60% parasitaemia. The mice in the prophylactic groups were passaged on day 15 post commencement of drug administration.

3.6.2 Drugs Tested

The following drugs were tested for their effects on the malaria parasites: lamivudine, zidovudine, nevirapine and stavudine as single drug therapies. Two different triple combinations of these drugs were also tested: lamivudine, zidovudine, nevirapine or lamivudine, stavudine, nevirapine (AIDS, 2005). The drugs were manufactured by Aurobindo Pharmaceuticals Limited, India. The date of manufacture and expiry date were as follows: lamivudine 05/2008-04/2010; zidovudine 04/2008-03/2010; nevirapine 07/2008-06/2010 and stavudine 12/2007-11/2009.

3.6.3 Drug Administration

lamivudine, zidovudine, nevirapine and stavudine at 4.3, 8.6, 5.7 and 0.9 mg/kg body weight respectively were administered as single drug therapies, through the oral route with the aid of a stainless steel metallic feeding canular after dissolving each drug in distilled water. The 2 different combinations of these drugs, lamivudine, zidovudine, nevirapine or lamivudine, stavudine, nevirapine were administered to each mouse as triple therapies. These drugs were given to 2 sets of mice, the first set on prophylactic basis and the second set on curative basis.

3.6.4 Collection of Animal Blood Specimen

A snip was made on the tail of the mice exposing some capillaries for the purpose of obtaining blood sample for malaria parasite counts.

3.6.5 Determination of Parasitaemia

The blood films obtained from the animal tail were stained with Geimsa stain and the percentage parasitaemia were determined by counting the number of parasitized red blood cells out of 1000 blood cells in 10 random microscopic fields.

3.6.6 Determination of Prophylactic Effect of Lamivudine, Zidovudine, Nevirapine and Stavudine on Malaria Parasitaemia

The prophylactic effect was investigated using the modified method by Peters (1965). Single drug therapies each of lamivudine, zidovudine, nevirapine, stavudine at 4.3, 8.6, 5.7 and 0.9 mg/kg body weight were administered as daily pretreatment to 4 groups of mice of either sex each weighing approximately 20g for 14 days. Each group contained 5 mice. On treatment day 15 (inoculation day 0), the mice were

passaged with *Plasmodium berghei* parasite. Treatment of the mice with the 4 drugs continued till day 26 post inoculation. Microscopic examination of the blood film was made to determine parasite concentration beginning from 72 hours post inoculation. Similarly, two groups of 5 mice each also had triple combinations of lamivudine / zidovudine / nevirapine and lamivudine / stavudine / nevirapine prophylactically. These were compared with a control group on chloroquine 25mg/kg body weight over 3 days and another on distilled water.

3.6.7 Determination of Curative Effect of Lamivudine, Zidovudine, Nevirapine and Stavudine on Malaria Parasitaemia

The modified method of Ryley and Peters (1970) was used. Eight groups of mice of either sex weighing approximately 20g each and containing 5 mice per group were passaged with *Plasmodium berghei* parasite. Microscopic examination of the blood film was made to determine the presence of malaria parasites and subsequently to assess parasite concentration beginning from 72 hours post inoculation. Out of the total mice for this experiment, 4 groups were administered with single drug therapies each of lamivudine, zidovudine, nevirapine, stavudine at 4.3, 8.6, 5.7 and 0.9 mg/kg body weight on a daily basis beginning from the day parasites were observed in the blood smears. Similarly, two groups of 5 mice each also had triple combinations of lamivudine / zidovudine / nevirapine and lamivudine / stavudine / nevirapine curatively. These were compared with a control group on chloroquine 25mg/kg body weight over 3 days and another on distilled water.

3.7 Determination of Sample Size for Human Experiment

The minimum number of patients with HIV required for the study was determined by using the appropriate formula for prevalence study thus:

$N = pq/E^2 \quad X\ (1.96)^2$ …….. (1)

Where n = Minimum number of patients required.

P = Maximum expected prevalence rate in percentage =4.4 (NACA, 2006)

Q = 100 – p.

E = Margin of sampling error tolerated, which is usually 5% with the confidence interval set at 95%.

1.96 = the factor obtained from the normal distribution table in connection with setting up the confidence interval at 95%.

Substitution in (1), gives:

$$n = \frac{4.4\ (100 - 4.4) \times 3.84}{5^2}$$

$$n = \frac{1615.2576}{25} = 65$$

3.8 Data Analysis

The data were analyzed using simple percentages and comparative analysis of proportions by Chi square or Students't-test as required. Test for significance was at 0.05 probability level. Correlation analysis was done where necessary. Results were expressed as mean ± Standard Error of Mean.

CHAPTER FOUR

4.0 RESULTS

4.1. Knowledge, Attitude and Practice In Respect Of Malaria Among Patients with the Human Immunodeficiency Virus Infection.

4.1.1 DEMOGRAPHY

A total of 469 patients attending the HIV Clinic in the Lagos University Teaching Hospital responded to the questionnaires. The age range was between 16 and 75 years, with a mean of 37.3 ± 0.5(SEM) years (Table 1). Approximately 58% were females, while 42% were males. Out of these respondents, 61% were married, while 28% were singles. The remaining respondents were separated, divorced or widowed. Out of the married patients, 49% had monogamous home setting, while 12% were from polygamous homes (Table 2). Among these patients, 234 (50%) had between 1 and 4 children, 48 (10.2%) had 5 to 8 children, 3 (0.6%) had over 8 children, while 56 (11.9%) did not have any child (Table 3). Most of the patients had secondary and tertiary education, 197 (42%) and 146 (31.1%) respectively, while 88 (18.8%) were educated up to primary school level and 20 (4.3%) had no formal education (Table 4). A total of 73.4% of the patients were employed (Table 5). Also, 81.9% of the married patients indicated that their spouses were employed while 14.6% had unemployed spouses (Table 6). Most of the patients fell within the N20,001-N50,000; N10,001-N20,000; N5,001-N10,000 monthly income groups respectively 17.2%, 10.9% and 6.6% of the patients (Table 7). Others earn monthly incomes of less than N5, 000 (3.4%); N50, 001 – N100,000 (4.5%) and over N100, 000 (1.7%).

Table 1: Age Distribution of Respondents to Questionnaires

Age(year)	**No of respondents**
16-25	40 (8.5%)
26-35	189 (40.3%)
36-45	131 (27.9%)
46-55	63 (13.4%)
56-65	19 (4.1%)
>65	6 (1.3%)
No Response	21 (4.5%)
Total	**469 (100%)**
Mean Age	**37.3 ± 0.5**
Sex	
Male	198 (42.2%)
Female	271 (57.8%)
Total	**469 (100%)**

Table 2: Marital Status and Home setting of respondents to questionnaires

Marital status	**No of respondents**
Single	133 (28.4%)
Married	287 (61.2%)
Separated	9 (1.9%)
Divorced	10 (2.1%)
Widowed	23 (4.9%)
No Response	7 (1.5%)
Total	**469 (100%)**
Home setting	
Monogamous	230 (49.%)
Polygamous	58 (12.4%)
Single	133 (28.4%)
No Response	48 (10.2%)
Total	**469 (100%)**

Table 3: Distribution of respondents according to number of their children

Number of Children	**No of respondents**
None	56 (11.9%)
1-4	234 (50%)
5-8	48 (10.2%)
>8	3 (0.6%)
No Response	128 (27.3%)
Total	**469 (100%)**

Table 4: Educational status of respondents

Education	**No of respondents**
No formal	20 (4.3%)
Primary	88 (18.8%)
Secondary	197 (42%)
Tertiary	146 (31.1%)
No Response	18 (3.8%)
Total	**469 (100%)**

Table 5: Employment status and occupation of respondents

Employment status	**No of respondents**
Employed	344 (73.4%)
Not employed	99 (21.1%)
No Response	26 (5.5%)
Total	**469 (100%)**
Occupation	
Professional	12 (2.6%)
Intermediate	36 (7.7%)
Non-manual skilled	41 (8.7%)
Manual skilled	73 (15.6%)
Partly skilled	146 (31.1%)
Unskilled	30 (6.4%)
No Response	131 (27.9%)
Total	**469 (100%)**

Table 6: Employment status and occupation of the respondents' spouses

Spouse's employment status	**Spouse's details**
Employed	235 (81.9%)
Not employed	42 (14.6%)
No Response	10 (3.5%)
Total	**287 (100%)**
Spouse's occupation	
Professional	7 (3.0%)
Intermediate	17 (7.2%)
Non-manual skilled	27 (11.5%)
Manual skilled	45 (19.2%)
Partly skilled	121 (51.5%)
Unskilled	6 (2.5%)
No Response	12 (5.1%)
Total	**235 (100%)**

Table 7: Family income of respondents

Family monthly income(Naira)	**No of respondents**
<5,000	16 (3.4%)
5,001-10,000	31 (6.6%)
10,001-20,000	51 (10.9%)
20,001-50,000	81 (17.2%)
50,001-100,000	21 (4.5%)
>100,000	8 (1.7%)
No Response	261 (55.7%)
Total	**469 (100%)**

4.1.2. KNOWLEDGE ABOUT MALARIA

4.1.2.1 Knowledge about the Cause of Malaria

Out of 494 respondents, 410 (83%) had correct knowledge about the cause of malaria. The remaining representing 17% erroneously cited things like drinking of bad water and exposure to the sun as the cause of malaria (Figure 3).

4.1.2.2. Knowledge of How Malaria Can Be Prevented.

A total of 235 (47.6%) respondents did not know how malaria can be prevented, while 53.4% did. Only 42 (8.5%) knew of prevention by insecticide treated bed nets, and 27.9% stated the use of non-insecticide treated bed nets as a preventive measure (Table 8).

4.1.2.3. Knowledge of Appropriate Drug For The Treatment of Malaria.

The commonest drug known to the respondents as appropriate antimalaria was sulphadoxine, this occurred in 141 (28.5%) cases (Table 9). Also, 58 and 45 respondents representing 11.7% and 9.1% respectively recognized Artemisinin based combination therapy and Artesunate as ideal antimalaria drugs. However, 161 respondents (32.7%) lacked the knowledge about antimalaria drugs.

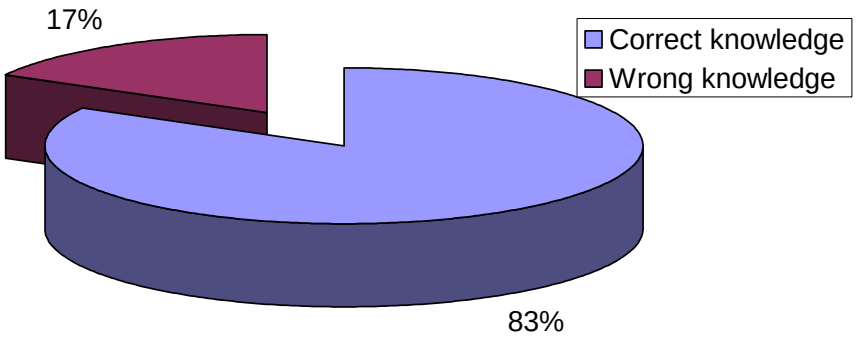

Figure 3: Knowledge about the cause of malaria

Table 8: Knowledge of how malaria can be prevented

Knowledge of prophylactic measures	**Number of respondents**	**Percentage (%)**
Insecticide treated bed nets	42	8.5
Non-insecticide treated bed nets	138	27.9
Drugs	54	10.9
Spraying	22	4.5
Door/window nets	3	0.6
Total correct knowledge	259	53.4
Wrong response	235	47.6
TOTAL	494	100

Table 9: Knowledge of drugs used to treat malaria

Drug choice	No of respondents	Percentage (%)
ACTS	58	11.7
Sulphadoxine	141	28.5
Chloroquine	76	15.4
Halofantrine	5	1.0
Amodiaquine	7	1.4
Proguanil	1	0.2
Artesunate	45	9.1
Wrong response	161	32.7
Total	**494**	**100**

ACTS= Artemisinin based Combination Therapy

4.1.2.4. Knowledge of Appropriate Dose of Antimalaria Drugs.

The appropriate dosage regimen of antimalaria drugs was known by 172 (34.8%) of the respondents while 322 (65.2%) lacked knowledge of the dosages of these drugs (Figure 4).

4.1.3. PRACTICE IN RESPECT OF MALARIA

4.1.3.1. Treatment Seeking Practice in Respect of Malaria

Out of the respondents, 88 (17.8%) indicated that they visit the HIV/AIDS clinic whenever they have malaria fever, while another 17.8% practice self-medication or receive treatment at home by members of their family (Table 10). Also, 66 (13.4%) respondents patronize drug hawkers, patent medicine stores or "neighbourhood nurse". In 10% of cases, pharmacy stores are visited. However, a greater proportion of these respondents, 75 (15.2%) and 92 (18.6%) respectively, attend the General hospitals or private hospitals whenever they suffer from malaria fever.

4.1.3.2. Use of Insecticide Treated Bed Nets For Prevention of Malaria

Insecticide treated bed nets were used by 244 patients representing 52%, while 225 (48%) patients did not use bed nets (Figure 5). The patients cited either difficulty with erecting the nets and/or heat generated by the nets as being the reasons why they did not use the bed nets.

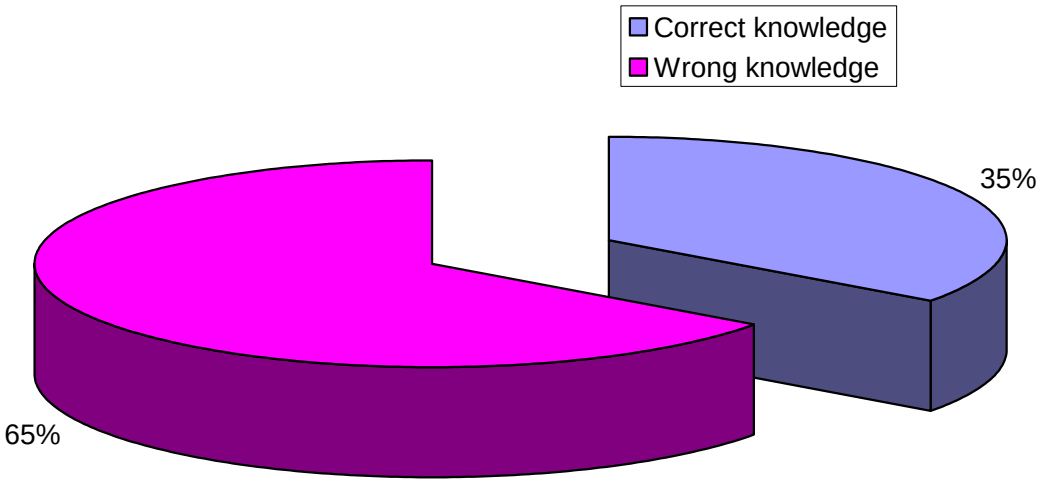

Figure 4: Knowledge of appropriate dose of antimalaria

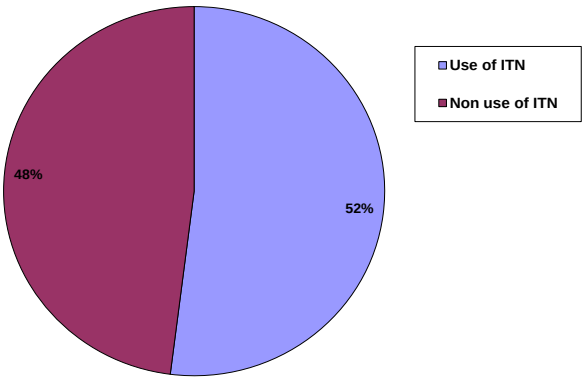

Figure 5: Use of Insecticide Treated bed Nets (ITN)

Table 10: Treatment seeking practice of respondents in respect to malaria

Facility/Point where treatment is sought	No of respondents	Percentage (%)
HIV Clinic in LUTH	88	17.8
Home care/Self-medication	88	17.8
Drug hawkers/Patent medicine store / Neighbourhood "nurse"	66	13.4
Traditional medicine doctor	3	0.6
Pharmacy Stores	50	10.1
General Hospitals	75	15.2
Private Hospitals	92	18.6
No response	32	6.5
TOTAL	**494**	**100**

4.1.3.3. Use of Prophylactic Antimalaria

Approximately 25.8% of these patients used antimalaria for prophylactic purposes while 74.2% did not . The commonest antimalaria used for prophylactic purposes was Sulphadoxine. Others were prognanil and pyrimethamine.

4.1.4. RELATIONSHIP BETWEEN THE SOCIO - ECONOMIC PATTERNS AND USE OF PROPHYLACTIC ANTIMALARIA

4.1.4.1 Relationship Between The Patient's Educational Level, Employment status, Occupation, Family Income and Use of Prophylactic Antimalaria

There was significant association between the patient's level of education and use of prophylactic antimalaria, p = 0.01 (Table 11). Those with secondary and tertiary education utilized prophylactic antimalaria the most, representing 47.9% and 39.3% respectively, of those who used antimalaria prophylactically. There was however no association between patients employment status, occupation, and monthly family income, p = 0.42, 0.30, 0.39 respectively.

Table 11: Association between some socio-economic variables of respondents and use of anti malaria for prophylaxis

Variable	Use of anti malaria for prophylaxis			X^2	df	p
	Use anti malaria	Do not use anti malaria	Total			
Education						
No formal	1 (5.0%)	19 (95.0%)	20 (100%)	12.5	3	0.01
Primary	14 (15.9%)	74 (84.1%)	88 (100%)			
Secondary	56 (28.6%)	140 (100%)	196 (100%)			
Tertiary	46 (31.9%)	98 (68.1%)	144 (100%)			
Total	**117 (26.1%)**	**331 (73.9%)**	**448 (100%)**			
Employment status						
Employed	85 (24.8%)	258 (75.2%)	343 (100%)	2.21	1	0.42
Not employed	32 (33.0%)	65 (67.0%)	97 (100%)			
Total	**117 (26.6%)**	**323 (73.4%)**	**440 (100%)**			
Occupation						
Professional	4 (30.8%)	9 (69.2%)	13 (100%)	6.12	5	0.30
Intermediate	8 (22.9%)	27 (77.1%)	35 (100%)			
Non-manual skilled	15 (36.6%)	26 (63.4%)	41 (100%)			
Manual skilled	14 (19.4%)	58 (80.6%)	72 (100%)			
Partly skilled	35 (24.0%)	111 (76.0%)	146 (100%)			
Unskilled	8 (26.7%)	22 (73.3%)	30 (100%)			
Total	**84 (24.9%)**	**253 (75.1%)**	**337 (100%)**			
Family monthly income (Naira)						
<5,000	3 (18.7%)	13 (81.3%)	16 (100%)	5.21	5	0.39
5,001-10,000	9 (29.0%)	22 (71.0%)	31 (100%)			
10,001-20,000	11 (21.6%)	40 (78.4%)	51 (100%)			
20,001-50,000	19 (23.7%)	61 (76.3%)	80 (100%)			
50,001-100,000	9 (42.9%)	12 (51.1%)	21 (100%)			
>100,000	1 (12.5%)	7 (87.5%)	8 (100%)			
Total	**52 (25.1%)**	**155 (74.9%)**	**207 (100%)**			

X^2 =Chi Square, df = degree of freedom, P=P value

4.1.4.2. Relationship between Spouse's Employment Status, Spouse's Occupation and Use of Prophylactic Antimalaria

There was no association between spouse's employment status, spouse's occupation and use of prophylactic anti-malaria, p = 0.40, 0.78 respectively (Table12).

4.1.5 RELATIONSHIP BETWEEN THE SOCIO - DEMOGRAPHIC PATTERN AND USE OF PROPHYLACTIC ANTIMALARIA

4.1.5.1 Relationship between The Age, Sex, Marital Status, Home Setting, Number of Children and Use of Prophylactic Antimalaria

There was significant association between the marital status of the patients and the prophylactic use of antimalaria, p = 0.01 (Table 13). Those who were single and those separated from their spouses used prophylactic antimalaria the most followed by those in the married group. They were 9.4%, 0.9% and 15.1% respectively. There was however no association between the age, sex, homesetting, number of children and the use of prophylactic antimalaria p = 0.07, 0.08, 0.95, 0.29 respectively.

4.1.6. Relationship between the Respondents' Socio - Demographic Characteristics and Use of Insecticide Treated Bed Nets.

There was no association between the respondent's age, sex, marital status, home setting, number of children and the use of insecticide treated bed nets , p = 0.57, 0.11, 0.54, 0.79, 0.31 respectively (Table 14).

Table 12: Association between spouses' socio - economic variables and use of anti malaria for prophylaxis

Variable	Use of antimalaria for prophylaxis			X^2	df	p
	Use of anti-malaria	Do not use anti-malaria	Total			
Spouse's employment status						
Employed	56 (24.0%)	177 (76.0%)	233 (100%)	0.72	1	0.40
Not employed	13 (31.7%)	28 (68.3%)	41 (100%)			
Total	**69 (25.2%)**	**205 (74.8%)**	**274 (100%)**			
Spouse's occupation						
Professional	1 (14.3%)	6 (85.7%)	7 (100%)	2.50	5	0.78
Intermediate	4 (23.5%)	13 (76.5%)	17 (100%)			
Non-manual skilled	7 (25.9%)	20 (74.1%)	27 (100%)			
Manual skilled	12 (26.7%)	33 (100%)	45 (100%)			
Partly skilled	29 (24.4%)	90 (75.6%)	119 (100%)			
Unskilled	0 (0)	6 (100%)	6 (100%)			
Total	**53 (24.0%)**	**168 (76.0%)**	**221 (100%)**			

X^2 =Chi Square, df = degree of freedom, P=P value

Table 13: Association between the socio-demographic variables of respondents and antimalaria use for prophylaxis

Variable	Use of anti malaria prophylaxis			X^2	df	p
	positive	negative	Total			
Age (year)						
16-25	14 (35.0%)	26 (65.0%)	40 (100%)	10.30	5	0.07
26-35	54 (29.0%)	132 (71.0%)	186 (100%)			
36-45	24 (18.3%)	107 (81.7%)	131 (100%)			
46-55	13 (20.6%)	50 (79.4%)	63 (100%)			
56-65	8 (42.1%)	11 (57.9%)	19 (100%)			
>65	2 (33.3%)	4 (66.7%)	6 (100%)			
Total	**115 (25.8%)**	**330 (74.2%)**	**445 (100%)**			
Sex						
Male	51 (25.8%)	147 (74.2%)	198 (100%)	0.06	1	0.80
Female	73 (27.2%)	195 (72.8%)	268 (100%)			
Total	**124 (26.6%)**	**342 (73.4%)**	**466 (100%)**			
Marital status						
Single	44 (33.1%)	89 (66.9%)	133 (100%)	14.6	4	0.01
Married	71 (24.9%)	214 (75.1%)	285 (100%)			
Separated	4 (44.4%)	5 (55.6%)	9 (100%)			
Divorced	1 (10.0%)	9 (90.0%)	10 (100%)			
Widowed	0 (0)	23 (100%)	23 (100%)			
Total	**120 26.1%)**	**340 (73.9%)**	**460 (100%)**			
Home setting						
Monogamous	48 (21.0%)	181 (79.0%)	229 (100%)	0.004	1	0.95
Polygamous	13 (22.4%)	45 (77.6%)	58 (100%)			
Total	**61 (21.3%)**	**226 (78.7%)**	**287 (100%)**			
Number of Children						
None	14 (25.9%)	40 (74.1%)	54 (100%)	3.79	3	0.29
1-4	60 (25.8%)	173 (74.2%)	233 (100%)			
5-8	7 (14.6%)	41 (85.45)	48 (100%)			
>8	0 (0)	3 (100%)	3 (100%)			
Total	**81 (24.0%)**	**257 (76.0%)**	**338 (100%)**			

X^2 =Chi Square, df = degree of freedom, P=P value

Table 14: Association between the respondent's socio-demographic variables and use of Insecticide Treated Bednets (ITN)

Variable	Use of ITN			X^2	df	p
	Positive	negative	Total			
Age (year)						

16-25	25 (62.5%)	15 (37.5%)	40 (100%)	3.88	5	0.57
26-35	95 (50.3%)	94 (49.7%)	189 (100%)			
36-45	66 (50.4%)	65 (49.6%)	131 (100%)			
46-55	37 (58.7%)	26 (41.3%)	63 (100%)			
56-65	9 (47.4%)	10 (52.6%)	19 (100%)			
>65	4 (66.7%)	2 (33.3%)	6 (100%)			
Total	**236 (52.7%)**	**212 (47.3%)**	**448 (100%)**			
Sex						
Male	94 (47.5%)	104 (52.5%)	198 (100%)	2.54	1	0.11
Female	150 (55.4%)	121 (44.6%)	271 (100%)			
Total	**244 (52.0%)**	**225 (48.0%)**	**469 (100%)**			
Marital status						
Single	66 (49.6%)	67 (50.4%)	133 (100%)	3.13	4	0.54
Married	157 (54.7%)	130 (45.3%)	287 (100%)			
Separated	3 (33.3%)	6 (66.7%)	9 (100%)			
Divorced	4 (40.0%)	6 (60.0%)	10 (100%)			
Widowed	11 (47.8%)	12 (52.2%)	23 (100%)			
Total	**241 (52.2%)**	**221 (47.8%)**	**462 (100%)**			
Home setting						
Monogamous	120 (52.2%)	110 (47.8%)	230 (100%)	0.07	1	0.79
Polygamous	32 (55.2%)	26 (44.8%)	58 (100%)			
Total	**152 (52.8%)**	**136 (47.2%)**	**288 (100%)**			
Number of Children						
None	23 (41.1%)	33 (58.9%)	56 (100%)	3.62	3	0.31
1-4	128 (54.7%)	106 (45.3%)	234 (100%)			
5-8	25 (52.1%)	23 (47.9%)	48 (100%)			
>8	2 (66.7%)	1 (33.3%)	3 (100%)			
Total	**178 (52.2%)**	**168 (47.8%)**	**341 (100%)**			

X^2 =Chi Square, df = degree of freedom, P=P value

4.1.7. RELATIONSHIP BETWEEN THE SOCIO-ECONOMIC PATTERNS AND USE OF INSECTICIDE TREATED BEDNETS.

4.1.7.1. Relationship between the Respondent's Socio- Economic Characteristics and the Use of Insecticide Treated Bed Nets.

There was no association between the patients' education, employment status, occupation, family monthly income and the use insecticide treated bed nets, p = 0.96, 0.13 , 0.28 , 0.56 respectively (Table15).

4.1.7.2. Relationship between Spouse's Socio-Economic Characteristics and Use of Insecticide Treated Bednets

There was no relationship between the spouse's employement status, spouse' occupation and the use of Insecticide Treated Bednets, p = 0.22, 0.42 respectively (Table 16).

.

Table 15: Association between the socio-economic variables of respondents and use of Inscticide Treated Bednets (ITN)

Variable	Use of ITN			X^2	df	p
	Use ITN	**Do not ITN**	**Total**			
Education						
No formal	10 (50.0%)	10 (50.0%)	20 (100%)	0.31	3	0.96
Primary	48 (54.5%)	40 (45.5%)	88 (100%)			
Secondary	105 (53.3%)	92 (46.7%)	197 (100%)			
Tertiary	75 (51.4%)	71 (48.6%)	146 (100%)			
Total	**238 (52.8%)**	**213 (47.2%)**	**451 (100%)**			
Employment status						
Employed	185 (53.8%)	159 (46.2%)	344 (100%)	2.32	1	0.13
Not employed	44 (44.4%)	55 (55.6%)	99 (100%)			
Total	**229 (51.7%)**	**214 (48.3%)**	**443 (100%)**			
Occupation						
Professional	10 (83.3%)	2 (16.7%)	12 (100%)	6.24	5	0.28
Intermediate	22 (61.1%)	14 (38.9%)	36 (100%)			
Non-manual skilled	19 (46.3%)	22 (53.7%)	41 (100%)			
Manual skilled	37 (50.7%)	36 (49.3%)	73 (100%)			
Partly skilled	77 (52.7%)	69 (47.3%)	146 (100%)			
Unskilled	16 (53.3%)	14 (46.7%)	30 (100%)			
Total	**181 (53.6%)**	**157 (46.4%)**	**338 (100%)**			
Family monthly income(Naira)						
<5,000	11 (68.8%)	5 (31.2%)	16 (100%)	3.94	5	0.56
5,001-10,000	15 (48.4%)	16 (51.6%)	31 (100%)			
10,001-20,000	32 (62.7%)	19 (37.3%)	51 (100%)			
20,001-50,000	46 (56.8%)	35 (43.2%)	81 (100%)			
50,001-100,000	11 (52.4%)	10 (47.6%)	21 (100%)			
>100,000	3 (37.5%)	5 (62.5%)	8 (100%)			
Total	**118 (56.7%)**	**90 (43.3%)**	**208 (100%)**			

X^2 =Chi Square, df = degree of freedom, P=P value

Table 16: Association between the spouse's socio-economic variables and use of Insecticide Treated Bednets (ITN)

Variable	Use of ITN					
	Use ITN	**Do not ITN**	**Total**	**X^2**	**df**	**p**
Spouse's employment status						
Employed	128 (54.5%)	107 (45.5%)	235 (100%)	1.49	1	0.22
Not employed	18 (42.9%)	24 (57.1%)	42 (100%)			
Total	**146 (52.7%)**	**131 (47.3%)**	**277 (100%)**			
Spouse's occupation						
Professional	6 (85.7%)	1 (14.3%)	7 (100%)	4.93	5	0.42
Intermediate	12 (70.6%)	5 (29.4%)	17 (100%)			
Non-manual skilled	14 (51.9%)	13 (48.1%)	27 (100%)			
Manual skilled	25 (55.6%)	20 (44.4%)	45 (100%)			
Partly skilled	63 (52.1%)	58 (47.9%)	121 (100%)			
Unskilled	3 (50.0%)	3 (50.0%)	6 (100%)			
Total	**123 (55.2%)**	**100 (44.8%)**	**223 (100%)**			

X^2 =Chi Square, df = degree of freedom, P=P value

4.2. Prevalence Rate of Malaria Parasitaemia

4.2.1 Prevalence Rate of Malaria Parasitaemia Among Patients Without Clinical Malaria Attending the Hiv Clinic In LUTH

4.2.1.1 Socio – Demographic Profile

There were 100 patients reviewed with Human Immunodeficiency virus infection who did not have clinically discernable malaria. Their age ranged between 16 and 60 years with a mean of 35.6±0.3 (SEM). Out of these, 78 were females while 22 were males (Table 17).

4.2.1.2 Prevalence of Malaria Parasitaemia

There were 15 patients whose blood smears were positive for malaria parasitaemia out of 100 sampled consecutively at the HIV clinic in LUTH, representing 15.0% (Figure 6).The presence of malaria was significantly more than control p = 0.000.

4.2.2. Relationship between Clinical Malaria and Parasitaemia among Patients with the Human Immunodeficiency Virus.

4.2.2.1. Socio-Demographic Profile

There were 100 patients with the Human Immunodeficiency Virus who were diagnosed as cases of malaria fever at the HIV clinic based on their clinical presentation (Table 18). These patients were aged between 19 and 63 years, with a mean age 38.7 ± 0.3 (SEM). There were 22 males and 78 females.

Table 17: Socio-demographic distribution of patients

Age(years)	No of respondents

16-25	10 (10.0%)
26-35	50 (50.0%)
36-45	28 (28.0%)
46-55	7 (7.0%)
56-65	5 (5.0%)
Total	**100**
Mean Age	**35.6 ± 0.3**
Sex	
Male	22 (22.0%)
Female	78(78.0%)
Total	**100 (100%)**

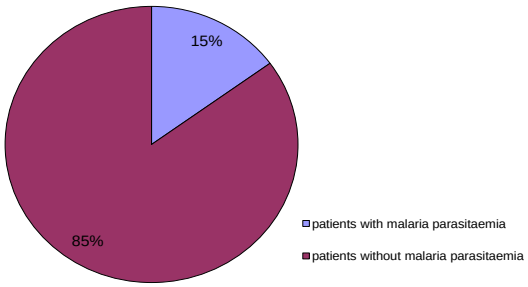

Figure 6: Prevalence rate of malaria parasitaemia among patients attending the HIV CLINIC in LUTH

Table 18: Socio - demographic distribution of patients with Human Immunodeficiency Virus infection diagnosed with clinical malaria

Age(year)	**Frequency**	**Percentage (%)**
16-25	8	8.0
26-35	37	37.0
36-45	30	30.0
46-55	19	19.0
56-65	6	6.0
Total	**100**	**100**
Mean age	**38.7± 0.3**	
Sex		
Male	22	22.0
Female	78	78.0
Total	**100**	**100**

4.2.2.2. Relationship Between Clinical Malaria And Presence of Malaria Parasitaemia

Out of the 100 patients diagnosed clinically as cases of malaria fever, 9 (9.0%) had malaria parasites in their blood film (Figure 7). The presence of malaria parasitaemia was significantly less than the control group P = 0.000.

4.3. Some Hematological Patterns of the HIV Patients

4.3.1 Distribution of the Patients According To Their Hemoglobin Concentration

Most of the patients (85.0%) had hemoglobin concentration of ≥10mg /dl while 15% had hemoglobin concentration below 10mg/dl (Table 19). Invariably, all who were in the lesser hemoglobin concentration group had concentrations of between 7 and 9 mg /dl.

4.3.2. Distribution of Hemoglobin Concnentration In Relation To Malaria Parasitaemia

There was no association between the patient's hemoglobin concentration and malaria parasitaemia. The few patients on drugs with ferrous supplements did not appear to have higher hemoglobin concentrations compared with those not on such supplements (Table 20).

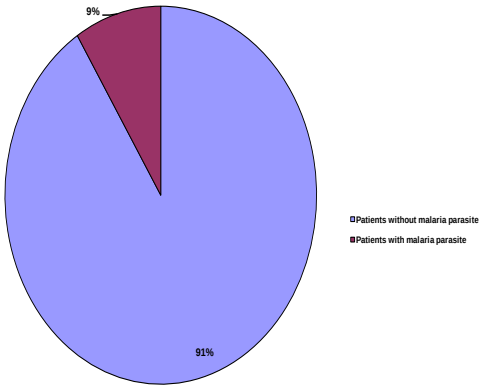

Figure 7: Relationship between clinical malaria and presence of malaria parasitaemia

Table 19: Distribution of respondents by Hemoglobin concentration

Hemoglobin concentration (mg/dl)	**HIV Positive**	
	Frequency	**Percentage (%)**
7-9	15	15.0
≥10	85	85.0
Total	**100**	**100**

Table 20: Relationship between the hemoglobin concentration and malaria parasite density of the HIV patients

Hemoglobin Concentration (mg/dl)	**Mean Malaria Parasite Density / µl of blood**
8	**336.0±0**
10	**208.0±0**
11	**706.7±235.9**
12	**484.8±146.2**
13	**474.7±282.8**
14	**414.7±76.6**
15	**380.0±170.9**

4.3.3. Distribution of the Patients According To Their CD_4 Counts

The largest single grouping of CD_4 count was >350 as found in 48.0% of the patients while the groups of 200-350 and <200 contained 29.0% and 23.0% of the patients respectively (Table 21).

4.3.4 Distribution of CD_4 Count In Relation To Malaria Parasitaemia

There was an increase in the average malaria parasite density between the group with the intermediate CD_4 count (200-350) and that with the lowest (CD_4 count <200) the values of 436.8 ± 166.5 / µl and 475.2 ± 146.8 / µl respectively. The group with the CD_4 count value of between 200-350 had the lowest average malaria parasite density (Table 22), while the group with the CD_4 count of > 350 had the highest average malaria parasite density of 496.9 ± 122.3 / µl .There was no significant difference between those with CD_4 <200 and 200 – 350, <200 and >350, 200 – 350 and >350 (P =0.954, 0.901, 0.692 respectively). Fifty percent of the patients in the groups with CD_4 values of <200 and 200 – 350 were on daily co – trimoxazole, while 12.5 % of those with >350 were on co – trimoxazole.

4.3.5. Distribution of Patients According To Their Blood Groups

Most of the patients were either in blood group O or A representing 49.0% and 29.0% respectively **(**Table 23**).**

Table 21: Distribution of the HIV positive patients according to CD_4 counts.

CD_4 Count	**Frequency**	**Percent (%)**
< 200	23	23.0
200-350	29	29.0
> 350	48	48.0
Total	**100**	**100**

Table 22: Relationship between CD_4 count, malaria parasitaemia and density

CD_4 count	**Patients with malaria parasite**	**Percentage on co – trimoxazoole (%)**	**Malaria parasite density / μl**
<200	6	50.0	475.2 ± 146.8 [a]
200-350	4	50.0	436.8 ± 166.5 [b]
>350	9	12.5	496.9 ± 122.3 [c]

P =0.954[a/b], 0.901[a/c], 0.692[b/c]

Table 23: Distribution of patients according to their blood groups.

Blood Group(ABO)	A	B	AB	O	Total
Frequency	29	20	2	49	100
Percentage **(%)**	29.0	20.0	2.0	49.0	100

4.3.6 Distribution of The Blood Groups In Relation To

Malaria Parasitaemia

The blood group with the highest average malaria parasite density was blood group O (Table 24), next was blood group B and the least blood group A, which were 487.2 ± 107.3 / µl, 274.4 ± 88.6 / µl and 187.2 ± 81.2 / µl respectively. There was a significant difference in the average malaria parasite density between those patients with blood groups O and A (P = 0.047).There was however no significant difference in the values got comparing patients who had blood group B with those in groups A or O (P = 0.874).

4.3.7. Distribution of Patients According To Their Genotypes.

Out of 100 patients, 70.0% had AA genotype, 23.0% had AS and 7.0 % were of the AC genotype. None had SS or SC genotypes (Table 25)

Table 24: Distribution of blood groups in relation to malaria parasitaemia

Blood group	**Malaria parasite density** / μl
A	187.2 ± 81.2
B	274.4 ± 88.6
O	487.2 ± 107.3

$P = 0.047^{o/a}$, $P = 0.874^{b/a}$.

Table 25: Distribution of patients according to their genotypes

Genotype	**Frequency**	**Percent (%)**
AA	70	70.0
AS	23	23.0
AC	7	7
SS /SC	0	0.0
Total	**100**	**100**

4.3.8 Distribution of The Genotypes In Relation To Malaria Parasitaemia

There were 9 patients whose blood smears were positive for malaria parasites, out of these, 7 (77.8%) were of the AA genotype, 1 was AS, the remaining 1 AC respectively. (Table 26).The mean malaria parasite density was highest among the patients with the AA genotype followed by those with AC and AS, the values were 356.4 ± 96.0 / µl, 34.7 ± 0 / µl, 26.7 ± 0 / µl respectively. However there was no significant difference between the mean malaria parasite density values of the patients with the genotype AA compared to genotypes AC and AS, P = 0.067, 0.057 respectively.

4.4. VIRAL LOAD OF THE HIV PATIENTS

4.4.1. DISTRIBUTION OF THE PATIENTS BY THEIR VIRAL LOAD

The viral load of 78.0% of the patients was less than 50,000 (Table 27). In 6.0% of these patients, it was between 50, 000 and 100, 000, while 16.0% of the patients had viral load of over 100, 000.

Table 26: Distribution of genotypes in relation to malaria parasitaemia

Genotype	No of patients with Malaria parasites	Malaria parasite density
AA	**7**	356.4 ± 96.0
AC	**1**	34.7 ± 0
AS	**1**	26.7 ± 0

P = $0.067^{AA/AC}$, $0.057^{AA/AS}$

Table 27: Distribution of the patients by Viral Load

Viral load	Frequency	Percent (%)
< 50,000	78	78.0
50,000 – 100,000	6	6.0
> 100,000	16	16.0
Total	**100**	**100**

4.4.2. DISTRIBUTION OF THE VIRAL LOAD IN RELATION TO MALARIA PARASITAEMIA.

Patients with a viral load of less than 20,000 constituted the largest group of 8 patients representing 53.3%; this was followed by those with viral load greater than 100,000 as found in 4 patients (26.7%). (Table28). Those with a viral load of 20,000 – 40,000 and 60,000 – 80,000 were 1 (6.7%) and 2 (13.3%) patients respectively. The greatest average malaria parasite density was in the group with viral load of less than 20,000, followed by the group of 20, 000 – 40,000; these 2 groups had malaria parasite densities of 487.0 ±125.6 / µl and 336 ± 0.0 / µl . The patients with viral load values of 60,000 – 80,000 and > 100,000 had average malaria parasite densities lower than those of the former. The average malaria parasite density of the patients with viral load <20,000 was significantly more than those with malaria parasite densities of 20,000 – 40,000 and 60,000 – 80,000, respectively P = 0.018, 0.021 .The patients with lower viral loads were on antiretroviral drugs while those with high viral loads were not on antiretroviral drugs.

4.5. CURRENT DRUG USE BY THE HIV PATIENTS.

4.5.1. CURRENT USE OF ANTIMALARIA DRUGS BY THESE PATIENTS.

Among these patients 19.0% were currently on antimalaria drugs taken in the last 28 days prior to study while 81.0% were not on any antimalaria agent in the last 28 days (Table 29).

Table 28: Distribution of the Viral Load in relation to Malaria Parasitaemia

Viral load	**Patients with MP(n = 15)**	**Average MP density / µl**
< 20,000[a]	8 (53.3%)	487.0 ±125.6
20,000 – 40,000[b]	1(6.7%)	336 ±0.0
40,000 – 60,000	-	-
60,000 – 80,000[c]	2(13.3%)	208 ±0.0
80,000 – 100,000	-	-
>100,000	4(26.7%)	255.0±128.2

P = 0.018[a/b], 0.021[a/c]

MP = Malaria Parasite

Table 29: Distribution of respondents by use of antimalaria

Use of antimalaria	**Frequency**	**Percent (%)**
On antimalaria	19	19.0
Not on antimalaria	81	81.0
Total	**100**	**100**

4.5.1.1 Distribution According To Types of Antimalaria Agents Taken by Respondents.

The commonest antimalaria agent taken was sulphadoxine / pyrimethamine, followed by co-trimoxazole, artemisinin based combination therapy, chloroquine, pyrimethamine, and proguanil respectively 31.6%, 26.2%, 21.1, 10.5, 5.3% and 5.3% (Table 30).

4.5.1.2. Relationship Between Current Antimalaria Agents and Malaria Parasitaemia.

Malaria parasites were detected in the blood films of 3 patients who had been on co-trimoxazole prescribed at the HIV Clinic for a duration of at least 28 days (Table 31). However, no malaria parasite was found in the blood smears of patients who had taken any of the antimalaria sulphadoxine / pyrimethamine, Artemisinin-based combination therapy and chloroquine within the same period.

4.5.2. DISTRIBUTION OF PATIENTS BY THE USE OF ANTIOXIDANTS

Out of the respondents, 32.1% were on antioxidants while 67.9% were not on any antioxidants (Figure 8)

Table 30: Distribution of respondents that are on antimalaria by the type of antimalaria

Type of antimalaria	HIV Positive, n=19	
	Frequency	**Percent (%)**
Sulphadoxine / pyrimethamine	6	31.6
Co-trimoxazole	5	26.2
Artesunate /ACTs*	4	21.1
Chloroquine	2	10.5
Pyrimethamine	1	5.3
Proguanil	1	5.3
Total	**19**	**100**

***Artemisinin based combination therapy**

Table 31: Relationship between antimalaria agents taken and Malaria parasitaemia

Antimalaria agent	**No of patients with malaria parasites**	**Average Malaria parasite density** / µl
Artesunate /ACT*	0	-
Chloroquine	0	-
Sulphadoxine pyrimethamine	0	-
Pyrimethamine	0	-
Proguanil	0	-
Co-trimoxazole	3	250.6 ± 42.7

***Artemisinin based combination therapy**

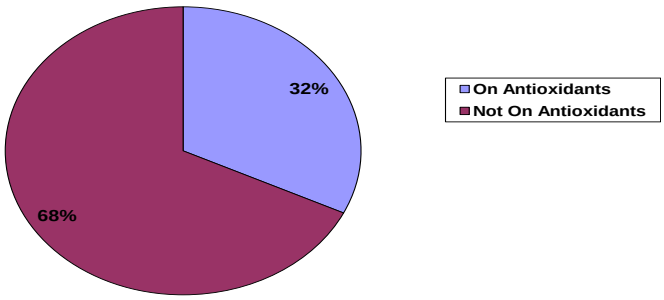

Figure 8: Distribution of patients by use of antioxidants

4.5.2.1. Distribution According To Different Types of Anti-Oxidants Used Currently.

The commonest anti-oxidant used was Heam 9 (64.3%) followed by vitamin C (16.7%) (Table32).

4.5.2.2. Relationship Between Current Antioxidant Use and Parasitaemia .

There was no association between the use of antioxidants by the patients and presence of malaria parasitaemia

4.5.3. DISTRIBUTION OF PATIENTS ACCORDING TO THE USE OF ANTIRETROVIRAL DRUGS

There were 65 patients on antiretroviral drugs while 35 were not on antiretroviral drugs. These represent 65.0 and 35.0% respectively (Figure 9).

4.5.3.1. Distribution of Patients According To The Types of Antiretroviral Drugs They Were Receiving

Most of those on antiretroviral drugs, 58.0% were on a combination of Nucleoside Reverse Transcriptase Inhibitors and Non-Nucleoside Reverse Ttranscriptase Inhibitors (NRTI / NNRTI). Others, 3.0% and 4.0% respectively were on Nucleoside Reverse Transcriptase Inhibitors and a combination of Nucleoside Reverse Transcriptase Inhibitors with Protease Inhibitors (Table 33).

Table 32: Distribution of the patients according to the types of antioxidants used.

Type of antioxidant	Frequency	Percentage (%)
Ginseng	4	9.4
Haem 9	27	64.3
Omega	1	2.4
Supradyne	1	2.4
Vit. C	7	16.7
Vit. E	2	4.8
Total	**42**	**100**

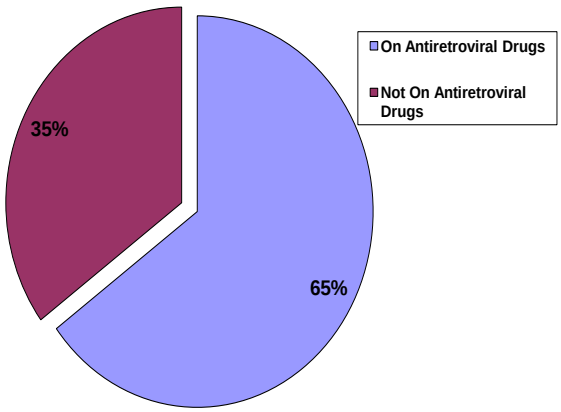

Figure 9: Distribution of patients according to the use of antiretroviral drugs

Table 33: Distribution of the patients according to the types of antiretroviral drugs used.

Use of antiretroviral	**Frequency**	**Percent (%)**
NRTI/NNRTI	58	58.0
NRTI	3	3.0
NRTI/PI	4	4.0
Total	**65**	**100**

NRTI =Nucleoside Reverse Transcriptase Inhibitors

NNRTI = Non-nucleoside Reverse Ttranscriptase Inhibitors

PI =Protease Inhibitors

4.5.3.2. Relationship Between Current Use of Antiretroviral Agents And Malaria Parasitaemia

Out of the patients on antiretroviral drugs, 9.6% had malaria parasitaemia while in 90.4% of cases, malaria parasites could not be demonstrated in the blood smears (Figure 10). There was significant association between the use of antiretroviral drugs and absence of malaria parasitaemia, $p = 0.006$.

4.6 ANTI PLASMODIAL EFFECT OF SOME ANTIRETROVIRAL DRUGS.

4.6.1 Prophylactic Effect of Lamivudine, Zidovudine, Nevirapine and Stavudine against *P. berghei* infection in mice

The mean malaria parasite density of mice on zidovudine was relatively lower, there was consistent decrease in the mean density with eventual total malaria parasite clearance by days 26 (Table 34). Also, the mice on lamivudine /zidovudine/ nevirapine combination had relatively lower mean malaria parasite density, which decreased and the parasites were eventually eliminated by day 20 (Table 35). The animals on lamivudine had an initial increase followed by a decrease and eventually, another period of increase in mean density from day 12 when the animals started dying off. The mice on stavudine had an initial increase followed by a constant level of mean malaria parasite density. Those on nevirapine had a remarkable period of decrease in mean parasite density up to day 8 after which the mean value remained approximately constant, while the lamivudine/stavudine/nevirapine group had an initial decrease followed by an increase in mean malaria parasite density.

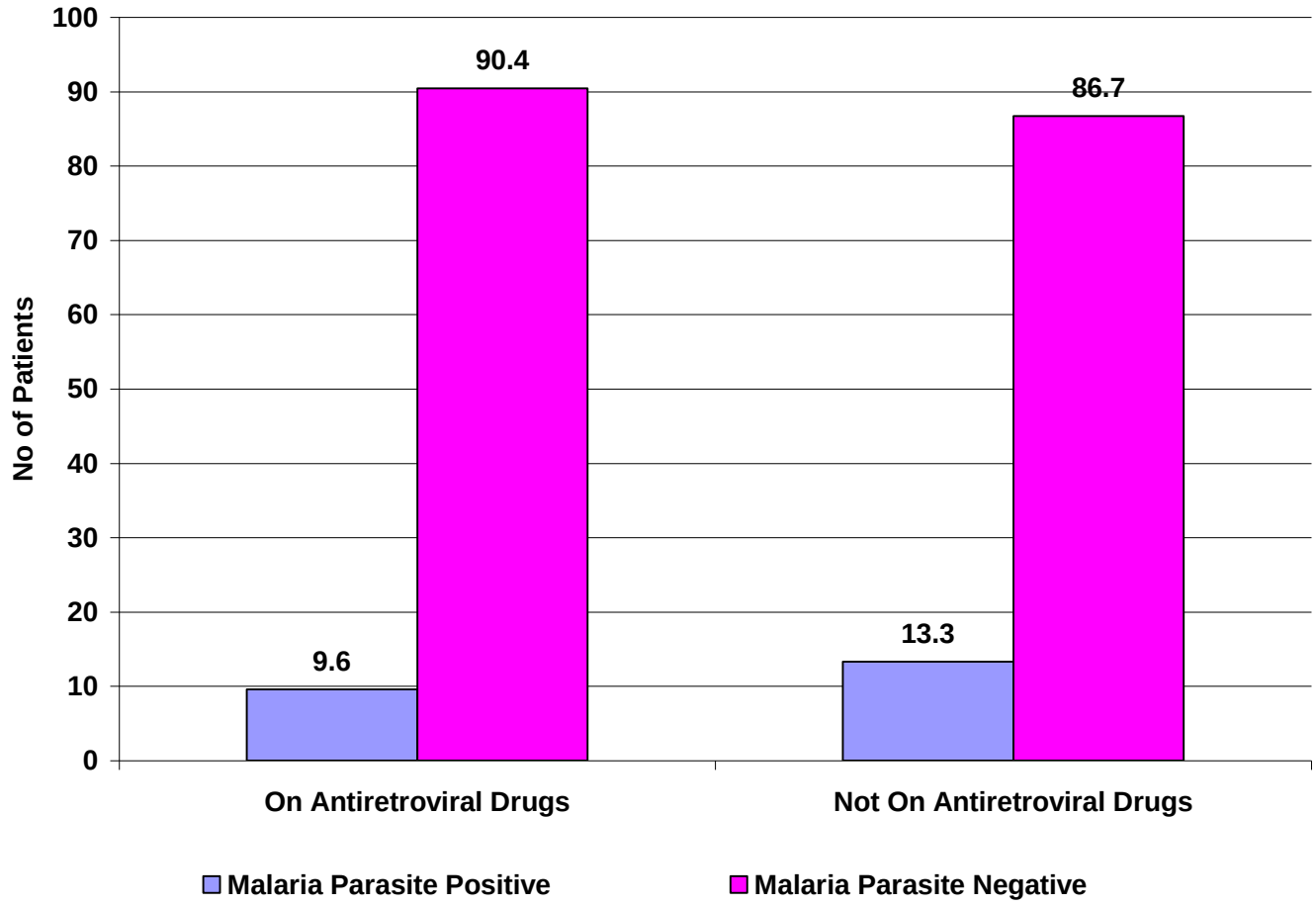

Figure 10: Relationship between current use of Antiretroviral drugs and malaria parasitaemia

Table 34: The effect of Prophylactic Lamivudine, Zidovudine, Nevirapine, and Stavudine on *Plasmodia berghei* infection in mice

DRUG GROUP	DAY 4	DAY 6	DAY 8	DAY 12	DAY 18	DAY 20	DAY 26
GP I (Distilled water)	20167.75 ± 14239.9	27113 ± 9469. 4	86411 ± 1986.03	86149 ± 27481.6	(5D)	(5D)	(5D)
GP II (Chloroquine)	71641.8 ± 42118.1	19451.3 ± 7667.8	22990.1 ± 13775.8	0	0	0	0
GP III (Lamivudine)	123966.02 ±44605.6	168014.26 ± 76389.2	44345.59 ±10339.5	59408.67 ±21637	97163.13 ±26271.5	(2D) 136073.42 ±52290.4	(5D)
GP IV (Zidovudine)	62474.85 ±14639.1	65792.43 ±31011.2	27429.47 ±9282.2	8196.19 ±4982.52	7256.15 ±5144.14	2601.33 ±0	0
GP V (Nevirapine)	367352.95 ±74933.2	(1D) 185726.01 ±48968.1	(1D) 94641.61 ±28210	(1D) 93067.5 ±28104	(2D) 59195.58 ±2052.29	(2D) 87571.44 ±5050.31	(5D)
GP VI (Stavudine)	0	11477.99 ±2786.24	64541.07 ±10544.7	41556.09 ±7668.2	(1D) 41497.16 ±8813.95	(5D)	(5D)

D =Number of Animals that died off

0 = No malaria parasite

Table 35: The Prophylactic effect of Lamivudine, Zidovudine, Nevirapine or Lamivudine, Stavudine, Nevirapine combinations on *plasmodia berghei* infection in mice

DRUG GROUP	DAY 4	DAY 6	DAY 10	DAY 12	DAY 14	DAY 18	DAY 20	DAY 26
GP I (Distilled water)	20167.75 ± 14239.9	27113 ± 9469. 4	86411 ± 19869.03	86149 ± 27481.6	(5D)	(5D)	(5D)	(5D)
GP II (Chloroquine)	71641.8 ± 42118.1	19451.3 ± 7667.8	22990.1 ± 13775.8	0	0	0	0	0
GP VII (LZN)	41138.1 ±3528.03	34554.95 ±11111.6	2727.27 ±747.27	2135.09 ± 963.42	1443.1±167. 55	(1D) 1243.1 ±462	(1D) 0	(1D) 0
GP VIII (LSN)	67987.69 ±6210.04	28428.75 ±828.8	47782.7 ±2610.4	(1D) 63386.74 ± 9045.37	(1D) 101786.76 ± 23643.7	(5D)	(5D)	(5D)

D =Number of Animals that died off

0 = No malaria parasite

LZN = Lamivudine, Zidovudine, Nevirapine.

LSN = Lamivudine,Stavudine, Nevirapine

4.6.2 Curative Effect of Lamivudine, Zidovudine, Nevirapine and Stavudine against *P. berghei* infection in mice

The mice on zidovudine had consistently decreasing mean malaria parasite density from day 8 and total malaria parasites clearance by day 26 (Table 36). The animals on lamivudine had an initial remarkable decrease of mean malaria parasite density terminating in an upward trend. The mice on stavudine had an initial decrease of mean parasite densities followed by an increase. The group on nevirapine had periods of decrease and increase of the mean malaria parasite density values. The group on lamivudine/zidovudine/nevirapine combination had relatively low mean values with complete malaria parasite clearance by day 13 (Table 37). The mice on lamivudine/stavudine/nevirapine had increasing mean parasite densities up to day 13 when the animals started dying off.

Table 36: The effect of curative regimens of Lamivudine, Zidovudine, Nevirapine, and Stavudine on *Plasmodia berghei* infection in mice

DRUG GROUP	DAY 4	DAY 6	DAY 8	DAY 12	DAY 15	DAY 18	DAY 20	DAY 26
GP I (Distilled water)	67794.5 ±9616.0	71866.25 ±7418.26	(1D) 98893.6 ±14834 .04	(1D) 136615.4 ±16393.9	(1D) 160320 ±20841.6	(3D) 195101.7 ±49686.9	(5D)	(5D)
GP II (Chloroquine)	116207.75 ±87038.8	37736 ±336	237.1 ±145.96	0	0	0	0	0
GP IX(Lamivudine)	184111.24 ±30237	52890.5 ±8113.5	31172.2 2 ±7914.1 6	(1D) 45398.03 ±13520.9	(1D) 56629.48 ± 12406.8	(1D) 67876.41 ± 23117.7	(3D) 33082. 71 ± 0	(5D)
GP X(Zidovudine)	62132.87 ±22816.1	74705.25 ±16397.7	30512.5 4 ±21880. 2	19949.0 ±6233.78	4703.2 ± 703.2	2821.4 ± 2342.44	621 ± 0	0
GP XI(Nevirapine)	162629.96 ±59030.6	55244.23 ±9003.39	75267.7 ±770.6	94072.9 ±32249.9	(2D) 51628.0 ± 528.6	(2D) 52415.37 ± 4182.57	(3D) 86584. 5 ±7966. 5	(5D)
GP XII (Stavudine)	103219.46 ±32230.5	65378.35 ±15985.0	27718.4 ±15221. 8	46290.06 ±14552.4	58238.98 ± 14360.5	(1D) 28246.43 ± 3672.51	(4D) 169655 .2 ±5333. 5	(5D)

D =Number of Animals that died off

0 = No malaria parasite

Table 37: The effect of Lamivudine, Zidovudine, Nevirapine or Lamivudine, Stavudine, Nevirapine combinations administered as curative regimens on *Plasmodia berghei* infection in mice

DRUG GROUP	DAY 4	DAY 7	DAY 10	DAY 13
GP I (Distilled water)	67794.5 ± 9616.0	71866.28 ± 7418.26	(1D) 99056.07 ± 19370.1	(1D) 78172.1 ±14852.7
GP II (Chloroquine)	116207.75 ± 87038.8	10152.5±6294.6	0	0
GPXIII (LZN)	31583.53 ± 6361.67	28783.75±5785.53	34446.53 ± 25018.9	0
GPXIV (LSN)	29784.95 ± 8507.11	28783.75 ± 11772.1	75814.48 ± 16265.6	(1D) 127456.05 ± 53275.1

D =Number of Animals that died off

0 = No malaria parasite

LZN = Lamivudine, Zidovudine, Nevirapine.

LSN = Lamivudine, Stavudine, Nevirapine

CHAPTER FIVE

5.0 DISCUSSION

This study revealed that the knowledge of prevention of malaria including the use of insecticide treated bed nets among these patients was poor. Only 53.4% knew how malaria could be prevented, while only 26.4% knew of prevention by bed nets. This shows the inability of the patients to translate the fact that they were given bed nets routinely in the HIV clinic to its use for prevention against mosquito bites and malaria fever. Also, approximately one-half (52%) of these patients used insecticide treated bed nets. In addition, while the knowledge and use of mosquito barrier for malaria prevention was not adequate, there was a tendency towards the use of antimalaria drug prophylaxis, a measure which is not a recommended standard in the management of malaria in HIV infected patients.Hence there is need to reinforce health education programmes directed at malaria prevention among these patients.

The main concerns about the interaction between malaria and HIV/AIDS are that the lowered immune status of patients with HIV predisposes them to developing malaria fever (WHO 2004a) and malaria fever enhances the multiplication of the virus which can lead to worse mobidity and mortality patterns. Also, the prognosis of malaria infection will be worse in such patients as is the case in immuno-compromised patients. This study however revealed that malaria may not occur as expected among patients infected with the Human Immunodeficiency Virus. This deviation from the expected malaria status of HIV patients noted in this study appears to be due to the role of the antiretroviral drug zidovudine. The results show that there was a significant association between the use of antiretroviral drugs and absence of malaria parasitaemia among the HIV Patients, $p=0.006$. This was further validated by the

result of the animal study, which showed total malaria clearance by zidovudine and lamivudine, zidovudine, nevirapine combination when administered as a prophylactic or curative regimen. Most patients in Nigeria are placed on lamivudine, zidovudine, nevirapine combination, since this is the first line drug therapy for HIV infection in the country hence the incidence of malaria parasitaemia and malaria fever among HIV virus infected patients in Nigeria and any other country, such as found in sub-Saharan Africa will be less than what has been projected to be over the last decade.

It should be noted that the previous projections, which postulated higher frequencies of malaria parasitaemia and malaria among HIV patients either did not consider the possible influence of antiretroviral drugs or utilized mathematical models based on some assumptions (Korenromp, 2005). Another finding which negates previous projections of expected high incidence of malaria among HIV patients is that there was no correlation between clinical diagnosis of malaria and demonstration of malaria parasitaemia in the blood smear of thes patients. Only 9% of those seen and diagnosed as cases of malaria in the HIV clinic had demonstrable malaria parasitaemia. The implications of these discoveries are far reaching. Apart from the necessity to consider the influence of antiretroviral drugs when defining the interactions between HIV virus infection and malaria, patients seen in the HIV clinic should have malaria parasite test before prescription of antimalaria drugs. Also, these patients need to be cautioned not to administer antimalaria drugs as self - medication, unless they have malaria parasite test. The latter is advisable because antimalaria drugs are sold as over-the - counter drugs and expected to be taken as self-medication in Nigeria, presently. These measures will lead to rational drug use and will curtail excessive use of antimalaria drugs, therefore reducing the incidence of untowards drug effects, development of resistance to antimalarials, wastage of otherwise useful funds on

inappropriate antimalaria therapy and omission of other casuses of fever in HIV infected patients. Ulitimately, the quality of care of these patients at the clinic will improve.

5.1 Polygamy Practised By Some of These Human Immunodeficiency Virus Infected Patients

The demographic characteristics of the home setting seen in this study where 12.4% or more than 1 out of every 5 married patients came from a polygamous home is an important consideration in the management of the HIV infection. This is because polygamy involves people having multiple sex partners, a situation implicated in the spread of HIV (UNHCR, 2005). Also, this underscores the need to make definite attempts at finding out if any patient with HIV has a polygamous home setting since such a situation is likely to impact on the finances of the family negatively and may therefore lead to difficulty in the family financing the patient especially in cases where the patient is unemployed or earns insufficient income.

5.2 Insufficient Knowledge and Poor Practice In The Prevention and Treatment of Malaria

Approximately 48% ,almost one - half of the respondents did not know any method of prevention of malaria and by extension could not even relate to the use of ITN or non ITN.This is discouraging especially in view of the fact that there have been campaigns on the use of ITN among these patients and to the general public through the use of the media in Nigeria .It suggests a low impact of these campaigns carried out over the last few years, a previous study of non – HIV infected individuals in Lagos State arrived at the same conclusion (Olayemi et al.,2004) Though more of these

respondents had the correct knowledge of possible drugs to use in the treatment of malaria, the proportion with the wrong knowledge, constituting 32.7% is still unacceptably high, since antimalaria drugs are classified as over-the-counter drugs in Nigeria and the average person may actually acquire these drugs without advice from a pharmacist or trained health workers, therefore, this proportion of the subjects may be prone to administering wrong dosages in the course of treating malaria fever. The knowledge of drugs used in treatment of malaria shows sulphadoxine as the commonest drug, a situation which still portrays sulphadoxine as the most likely drug to be used by these patients, if they are to administer self - medication.This still confirms sulphadoxine as the likely commonest antimalaria in use presently as asserted by other authors (Schoepflin *et al.,* 2008). Unfortunately the second commonest drug cited in this study by the respondents is chloroquine while arteminisin based combination therapy and artesunate were the third options, a pointer to the need for campaigns to enlighten this group of patients on the current drug recommendation for treatment of malaria fever in Nigeria.

The tendency toward the use of self-medication has been associated with increase in the level of education of the general public (Knapp *et al*., 1981). This tendency was seen among the patients in this study in the use of antimalaria for prophylactic purpose. There was a significant increase in the use of antimalaria prophylaxis as the patients' educational level increased. Such a trend is normally due to the fact that the drug user has more knowledge about the drug compared to others in the society who do not self-medicate. This may give them more confidence to use the drugs compared to the average person in the society. While self-medication may be encouraged in some instances particularly when there is adequate knowledge of the use of the drugs

in question (Lasagna, 1970), at other times such knowledge is absent and self-medication is to be discouraged.The possibility that a person on self-medication may lack necessary information on the use of the drug is a reality, a fact that can be supported by the discovery in this study that 65.2% of the respondents had wrong knowledge of the dosage regimen of antimalaria drugs. Incidentally, the use of antimalaria prophylaxis by patients is wrong because HIV is not a known indication for antimalaria prophylaxis; this practice is likely to exert more pressure on the antimalaria drugs and therefore enhance resistance to these drugs. There was significant association between the marital status of the patients and the use of anti-malaria prophylaxis by self-medication. The use of antimalaria was highest among those who were separated from their spouses, followed by those who were single. The reason for this observation is not clear, though these two groups share the fact of being without a spouse in common.

Only 52% of the patients use insecticide-treated bed nets. The rate of the use of insecticide-treated bed nets is lower than expected, considering the fact that these patients were routinely given these bed nets at no charge. The factors responsible for such poor utilization of the bed nets, which have proven to be of immense benefit (Goodman et al., 1999; Cochrane Collaboration, 2006), among such include; heat generated by the nets and the difficulty in setting up the nets where the patients could not find points on the wall of their rooms on which to hang the nets. There was also the reluctance to construct hanger points on the walls in some cases because their landlords would frown at any construction work on the walls. It is necessary to campaign with practical demonstration among these patients on ways of setting up the mosquito nets using wood poles and ropes or screw which will keep the insecticide

treated bed nets in place such that they do not need to bother setting up hanger points on the walls. The monthly Saturday meetings organized by this group of patients in LUTH can be used as an avenue for a workshop aimed at this campaign.

5.3 Poor Correlation Between Clinical Malaria And Parasitaemia

There was a poor correlation between the clinical diagnosis of malaria fever and laboratory demonstration of malaria parasite in the patients' blood stream. Only 9 (9%) of the 100 patients diagnosed clinically as cases of malaria were confirmed with malaria parasite positive blood smears. The significance of this is that malaria fever is likely to be over treated among patients attending the HIV clinic unless they are screened for malaria parasitaemia, a procedure that is usually absent in this environment. The practice of diagnosing patients clinically as cases of malaria and treating them with anti-malaria without laboratory confirmation is normally extended to HIV patients. There are two reasons why such empirical treatment for malaria among patients may occur more commonly than expected; these patients would have been bled at the regular clinic before presenting to the doctor, so there is normally a reluctance in asking for blood samples to be taken again, also considering the fact that the HIV can be spread through blood contamination, bleeding of these patients is usually restricted and health personnel are usually reluctant to obtain blood samples from them except when absolutely neccesary. However, the result obtained in this study underscores the necessity to make diagnosis of malaria fever in these patients relying on malaria parasite demonstration in their blood smears. Other workers also advocate the use of laboratory procedures to diagnose malaria in order to achieve more accurate diagnosis and promote rational drug use (WHO, 2004c) Otherwise,

treatment based on clinical diagnosis will result in over treatment for malaria and its consequences which include emergence of resistance to anti-malaria drugs, wastage of medical and financial resources on such treatment and avoidable adverse drug reactions.

5.4 Emerging Resistance To Antimalaria Drugs

It is interesting to note that a significant proportion of these patients, 19.0% were currently on drugs with antimalaria action. This was significantly more than controls P=0.0001. Approximately 75.0% of those on anti-malaria agents were on regular anti-malaria drugs such as artemisinin based combination and chloroquine. These were bought by the patients as self-medication. Others procured the drugs upon the advice of pharmacists or patent medicine sellers. These patients took these drugs because they had symptoms which they felt were due to malaria. The remaining half was on cotrimoxazole an antibacteria agent, prescribed by physicians at the HIV clinic for chemoprophylaxis.The latter is in congruence with earlier reports by Anglaret *et al.*, 1999; Wiktor *et al.*, 1999. Co-trimoxazole has been known to exert antimalaria action (WHO 2004a, Mermin *et al.,* 2005) and the WHO has recommended that pregnant women on prophylactic co- trimoxazole should not receive Intrapertum Presumptive Therapy with sulphadoxine (WHO 2004a, WHO, 2006).The reason for this is that there is the possibility of development of cross resistance between co-trimoxazole and sulphadoxine evident at the molecular level (Lyer et al., 2001).Hence, pregnant patients on daily co-trimoxazole are prone to having sulphonamide resistant malaria parasitaemia whenever infected with malaria. Also, the wide spread use of sulphadoxine which is the commonest antimalaria currently in use (Schoepflin et al., 2008) will be an added factor in the development of resistance. Sulphadoxine

/pyrimethamine combination is prescribed to pregnant women as prophylaxis against malaria as a single dose given during the first, second, third trimesters or during the second and third trimester for the HIV infected women or non HIV infected women respectively (WHO, 2004b; Hetzel, 2007; van Eijk, 2004), also it is used as part of some artemisinin based combination therapy. Previously, it was employed as a curative drug against malaria in Nigeria given as a single dose and it has been an over-the-counter drug for years. The ease of it being a single dose therapy and its relatively low cost will definitely enhance its relative common use as a self-medication agent. This study illustrates the drug as the commonest antimalaria agent utilized without doctors' prescription by the HIV infected patients at the LUTH. It is obvious that there is drug pressure on sulphadoxine considering its rate of use and significant malaria parasite resistance to the drug may emerge soon. Further, indications of possible resistance to sulphadoxine are the cases of 3 HIV patients in this study who had been on co-trimoxazole as prophylaxis against opportunistic infections whose blood samples were positive for malaria parasite.This study revealed again one of the setbacks with chloroquine utilization which contributed to its loss of efficacy over the years, the problem of inappropriate dosage (Yousif and Adeel, 2000; Okafor and Odeyemi, 2009; Aina et al., 2009). Virtually all the patients who used this drug bought suboptimal dosages and therefore administered wrong dosages. This illustrates the problem that can be encountered when drugs are not properly packaged as is found in the case of chloroquine. Drugs should be properly packaged to enhance dosage administration and to exclude procurement of suboptimal doses. This is particularly advisable when multiple doses of a drug are needed for complete cure of an ailment. In addition, it is unexpected that chloroquine will still be sold as an antimalaria drug to patients in Nigeria. There has been a policy shift from the use of

chloroquine in Nigeria along with other African countries in the last few years (WHO, 2007). The purchase and use of this drug by some patients in this study shows that the policy shift is still circumvented. It is necessary to ascertain how common the use of chloroquine is presently in Nigeria and implement necessary steps to ensure full compliance with the Federal Government policy on the use of chloroquine.

The use of antimalaria agents by the patients with HIV infection was not significantly associated with absence of malaria parasitaemia among these patients when subjected to statistical analysis. This suggests that for these patients, malaria parasitaemia may not be determined by the use of antimalaria drugs. If the use of antimalaria drugs by these patients did not influence the occurrence of malaria parasitaemia among these patients, then in all probability, the use of this group of antimicrobes was a wasted effort, the drug was irrationally used as is usual when appropriate diagnostic criteria is not employed before the commencement of medication, in this case, the use of antimalaria therapy. Hence there is need for caution to be exercised in the use of conventional antimalaria drugs.Considering the fact that there are quite a number of infections in patients with HIV which could lead to fever, there is the need to confirm malaria parasitaemia with a diagnostic laboratory test before commencing antimalaria therapy. Patients with the Human Immuno-deficiency Virus in Nigeria may need to be exempted from the routine self- treatment of malaria based on the perception of fever and allied features of malaria. Such drug use practice is in line with observations and suggestions of other workers (WHO, 2004c; Masika *et al.*, 2006) and prevents the formation of resistance to antimalaria drugs (WHO, 2006).

5.5 Antioxidant Use Not Associated With Malaria Parasitaemia

Among the patients studied, 32.1% were on antioxidants, most of who were on high potency antioxidants such as Ginseng and Haem 9. Antioxidants administration in the form of retinol has been linked with increased host resistance to malaria (Serghides and Kain, 2002; Shanker *et al.,* 1999). *In vitro* studies have demonstrated the antiplasmodial activity of retinol (Davis *et al.*, 1998; Hamza *et al.*, 2004). Patients with HIV infection are sometimes placed on antioxidants by their physicians (Greenspan and Aruoma, 1994) with the hope of reducing oxidative stress which tends to occur in them.Such use of antioxidants in patients with HIV may not have an impact on malaria parasitaemia among these patients as potrayed by this study which showed no association between malaria parasitaemaia and the use of antioxidants.

5.6 Reversal of Malaria-induced Human Immunodeficiency Viral Replication By Antiretrovirals

Malaria is said to increase the viral load of patients with HIV infection (UNICEF, 2003) and therefore is a potential enhancer of HIV spread because the infectivity rate is likely to be increased during periods of malaria fever. Incidentally, this study shows no significant association between the presence of malaria parasites in the patients blood smears and the viral load.The group with the least viral load had a greater mean malaria parasite compared with the group with the largest viral load, these were viral load values of < 20,000 = 487.00 ± 125.6 / µl and viral load > 100,000 = 255.00 ± 128.2 / µl respectively.

This trend is in opposition to the assertion by other workers that malaria parasitaemia increases viral load.However, the use of antiretroviral drugs appears to be a factor in determining the viral load increase among the patients with malaria in this study as patients with malaria parasitaemia and low viral load were on antiretroviral drugs during the period, while those who had high viral load were not on antiretroviral drugs.Thus increase in viral load and therefore HIV infectivity of these patients when there is malaria infection may be modulated by the use of antiretroviral drugs. This underscores the importance of ensuring that HIV infected patients on antiretroviral agents in malaria infested regions adhere to their drug therapy. Every available resource should be utilized to ensure availability of these drugs to the patients. Also, it may be necessary to revisit the guidelines which stipulate the criteria for placing HIV patients on antiretroviral drugs. These guidelines exempt a group of patients from antiretroviral medication; however, considering the casual relationship between these two diseases in malaria endemic regions it may be appropriate to place all patients in these regions on antiretroviral drugs.

5.7 Lowered CD_4 Count Not Necessarily Correlated With Malaria Tendency In Human Immunodeficiency Virus Infection

The CD_4 count is an important indicator to ascertain the level of immunity and well-being of HIV infected patients (Balter, 1997; Baum *et al.*, 1995; Audu *et al.*, 2005). It has been stated that the lowered immune status of HIV infected patients predisposes them to malaria infection (Good and Doolan, 1999; Moore *et al.*, 2000) and that as their immune level drops, patients will tend to have more frequent malaria infections and malaria fever (Whitworth *et al.*, 2000; French *et al.*, 2001). The average malaria parasite density in the group with the lowest CD_4 count (<200) was higher than that of

the intermediate group which contained patients with CD_4 count of between 200 and 300. These had average malaria densities of 475.2 ± 146.8 / µl and 436.8 ± 166.5 / µl respectively. This trend suggests that there is a tendency of malaria parasitaemia to increase among the HIV patients as the CD_4 count and thus the immune status of these patients diminish, though this association was not significant statistically($p = 0.954$). The group, with a CD_4 count of >350 however, had an average malaria parasite density of 496.9 ± 122.3 / µl which was unexpectedly higher than found with the 2 groups of patients with lower CD_4 counts.The mean malaria parasite density of the patients with the highest CD_4 count of >350 was unexpectedly the highest.The reason is not so clear, but the fact that a greater proportion of the patients with CD_4 values <200 and 200-350 were on daily co-trimoxazole, a drug known to possess antimalaria property may be an explaination for this observation.

5.8 Malaria In Human Immunodefciency Virus Infected Patients Not Associated With Anemia

Anemia is linked with HIV infection (Nielsen *et al.*, 2005; Kowalska *et al.*, 2007), while treatment with antiretroviral drugs is known to reverse this trend in some patients, other patients despite treatment progress to anemia subsequently (Kowalska *et al.,* 2007). Anemia is also known to be associated with progression to AIDS and death among HIV infected patients (Kowalska *et al.,* 2007). Malaria parasite in the other hand, invades the red blood cells lysing them, hence there is a tendency towards anemia which has been documented in malaria disease (Menendez *et al.,* 2000; Mohan *et al.,* 1995, Abdalla *et al.*, 1980; Abdalla and Wickramasinghe, 1988). Anaemia, during malaria infection is most frequently seen in young children and pregnant women (Menendez *et al.,* 2000). This occurs in acute, as well as chronic

repeated malarial infection. The underlying causes of severe malarial anemia are likely multifactorial. Extravascular and intravascular hemolysis of both infected and uninfected erthrocytes play a role. Changes occur in the clearance of these cells (Mohan *et al.*, 1995), while non infected blood cells are destroyed in the spleen during acute infection. This leads to hemolysis and depletion of iron stores. Bone marrow suppression also plays an important role in the pathogenesis of malarial anaemia. The normal response to hemolytic anemaia is enhanced secretion of erythropoietin leading to stimulation of erythropoiesis with malaria. During acute infections, abnormalities are seen in erythroid progenitors (Abdalla and Wickramasinghe, 1988), while dyserythropoiesis is observed in chronic infection (Abdalla *et al.*, 1980).

Considering the implication of malaria parasitaemia and anemia in patients with HIV patients, theoretically low hemoglobin concentration levels may occur. However, there was no association between hemoglobin levels of the patients in this study and malaria parasitaemia even among those who were not on any ferrous supplement. The values of hemoglobin concentration of below 10g/dl found among 2 patients could not be correlated with the malaria parasite density of these persons. The non correlation of anaemia with malria in these patients with Human Immunodeficiency Virus infection is similar to another study carried out in Tanzania (Nielsen *et. al.*, 2005). This means that more frequent malaria infection or higher malaria parasite density among patients with HIV may not enhance anemia.

The AC and AS genotypes are postulated to have arisen as a result of malaria assault. It has been deduced that, over time, there has been switches from A A genotypes to the A C or AS genotypes, along the lineage of people in malaria endemic regions

(Rihet *et al.,* 2004), in order to resist malaria because of the relative protection the C and S haemoglobin types offer against malaria (Rihet *et al.,* 2004; Hill *et al.,* 1991; Aidoo *et al.*, 2002).

The SS and SC genotype combinations are not so compatible with survival in life and people with these two combinations tend to die before adulthood, so it is not surprising that this study which recruited adults recorded no HIV infected patients with the SS and SC genotypes because such persons are relatively not many in the society. Also, the commonest genotype among the patients recruited into this study is the AA representing 70.0%, this is not surprising since the patterns of genotypes of patients with HIV infection should reflect the patterns of genotypes found in the society. The same reason accounts for those with AS found to be the next common genotype combination. The HIV infected patients with the genotype AA constituted the highest number of patients with malaria parasites in their blood smears. These were 7 (77.8%) in number out of 9 patients who had malaria parasitaemia. Each of the remaining 2 patients with malaria parasitaemia was either AS or AC. The implication of this finding is that there is a tendency of these HIV infected patients with genotype AA to have more malaria parasitaemia compared with AS. Although statistically this tendency is not significant, it is similar to the trend among persons not infected with HIV who have been found to have more malaria parasite density when their genotype is AA compared with AS (Marsh *et al.,* 1989; Thompson, 1963; Allison,1954) or AC (Rihet *et al.*, 2004) individuals . The explanation is that when either hemoglobin S or C is infected by malaria parasites, there is consequent elimination of such parasites because of the metabolic activity of the parasite and clearance of the red blood cell by the reticulo-endothelial system (Friedman, 1978).

5.9 Anti Plasmodial Activity of Antiretroviral Drugs

The use of antiretroviral drugs by patients with HIV infection in this study was associated with absence of malaria parasites in the patient's blood smear. This study suggests that HIV patients on antiretroviral drugs may have a diminished tendency towards malaria parasitaemia and by extension are less likely to develop malaria fever. The implication of this suggestion of decreased malaria morbidity among HIV infected patients on antiretroviral drugs is that there may be a need for a new approach towards defining the relationship between HIV and malaria. HIV infected patients on antiretroviral drugs may need to be cautioned about self-medication with antimalaria. Treatment of malaria should be only after consulting a doctor. The doctor will need to test the blood sample for malaria parasite before prescribing any antimalaria knowing that this group of patients has some degree of protection against malaria. It will be necessary to find out the malaria status of HIV infected pregnant patients on antiretroviral drugs. This will help ascertain the pros and cons of the Intermittent Presumptive Therapy in pregnant women and help us conclude whether or not there is need to give sulphadoxine prophylactically among HIV infected pregnant women.

The discovery that the HIV infected paients on antiretrovirals in the present study had significantly less malaria parasitaemia than those not on ARVs led to the experiment to verify the effects of these drugs on malaria parasitaemia in mice, hence the findings that zidovudine and the triple combination of lamivudine, zidovudine and nevirapine cleared plasmodia berghei parasites completely from the blood of the mice. The protease inhibitors, saquinavir, ritonavir and Indinavir have been proved experimentally to possess antiplasmoia activity before now (Skinner Adams *et al.*,

2004). Incidentally, the conclusion of another study in children is that the first line antiretroviral drugs used in Nigeria do not possess antimalarial activity (Adetifa *et al*., 2008). However, this study has established the antimalarial property of lamivudine, zidovudine, nevirapine combination which is the first line regimen used in Nigeria for treatment of patients with the Human Immnodeficiency Virus (HIV) infection. Nigeria is located in the sub-saharan regions which is a malaria endemic region (WHO, 2006). By implication, such patients in Nigeria should tend to suffer frequent and more severe attacks of malaria, a burden which has caused serious concerns for the following reasons:

- Co-existence of HIV and malaria infections would definitely impact the health and socio-economic status of infected individals.
- Malaria paraitaemia and malaria fever increase the viral load of HIV patients. This can lead to amplified HIV disease progression and also increase in infectivity.
- In the event of patients with HIV infection having more episoes of malaria attacks and malaria parasitemia, there will be an upsurge in the transmission of malaria even to non-HIV infected individuals (Whitworth *et al*., 2000) and the global epidemiology of malaria will become worse.

Hence, the importance and advantage of this finding that a first line therapy of HIV disease eliminates malaria parasities. This pharmacotherapy should therefore be given to HIV infected patients in malaria prone regions of the world unless there is a reasonable contra-indication. The control of malaria as a consequence of antiretroviral drug use in HIV patients is one of the gains of international philanthropic programmes directed against HIV/AIDS (Oshinaike *et al*., 2009) zidovudine and lamivudine, zidovudine, nevirapine triple combinations were found in this present study to

eliminate malaria parasitaemia when administered prophylactically and as curaive therapy. This implies that HIV patients on these drugs have a prophylactic advantage against malaria fever and patients with malaria parasitaemia newly diagnosed as cases with HIV may not progress to frank cases of clinical malaria once placed on these antiretroviral therapies. It has been suggested that HIV infected patients should be placed on prophylactic antimalaria (Whitworth, 2006). The use of these drugs with antiplasmodial activity may make such an argument unnecessary.

It is important to re-evaluate the practice of Intrapartum Preventive Therapy with the antimalaria, sulphadoxine in view of the antimalaria action of the antiretroviral drugs shown in his study, whenever tthese drgs are used to treat such patients. It is important to review previous studies on the incidences of malaria parasitaemia prevelance among HIV infected persons adjusting for the possible antipalsmodial effects of zidovudine, and lamivudine, zidovudine, nevirapine combinations in these patients. This may unravel the conflict possed by some authors that HIV infection may not result in an upsurge of malaria fever or malaria parasitaemia (Chandramohan and Greenwood, 1998; Muller and Moser, 1990). The epidemiology of malaria among HIV infected patients need to be redefined particurlary since virtually all epidemiological frames constructed so far are computer simulated models and not physical sampling/ research work (Korenromp *et al.*,2005). Another implication of the discovery in this study that zidovudine and its triple combination with lamivudine/nevirapine eliminates malaria parasite is that since malaria will be well controlled in HIV patients placed on these antiretrovirals, the expected malaria induced increase in Human Immunodeficiency viral load will not occur. The patients' illness will therefore not progress rapidly and infectivity will be better controlled

SUMMARY OF FINDINGS:

The following findings were demonstrated in this work:

1. The knowledge of prevention of malaria, and drug dosages in treatment of malaria was poor among these patients in spite of the fact that these drugs are obtainable as over - the - counter drugs in Nigeria.

   In addition, a little less than one-half (48%) of the patients reviewed sought treatment for malaria from unacceptable sources.
2. The use of insecticide treated bednets among these patients was lower than expected.There was no correlation between the use of insecticide treated bednets and malaria parasitaemia. Also, the use of antimalaria prophylaxis, a practice without medical basis was found to be common among patients reviewed.
3. The prevalence of malaria parasitaemia among the HIV infected patients without clinical malaria was 10%.
4. There was a poor correlation between the clinical diagnosis of malaria and the presence of malaria parasites in the blood smears of the patients reviewed.
5. Some patients on daily co-trimoxazole prophylactic therapy have malaria parasitaemia, an indication of emerging resistance to this drug and possibly cross resistance to the sulphadoxine/pyrimethamine combination.
6. *Plasmodium berghei* malaria parasitaemia in mice were completely eradicated by zidovudine, and the triple drug combination of lamivudine, zidovudine, nevirapine.

CONTRIBUTIONS TO KNOWLEDGE

The findings in this research project have provided the following contributions to knowledge:

1. Zidovudine regimen eliminated malaria parasites; this will obviously alter the prevalence of malaria parasitaemia in sub-Saharan Africa.
2. Malaria is over treated at the HIV Clinic in Lagos University Teaching Hospital (LUTH). This underscores the need for malaria parasite test.
3. There is evidence of emerging resistance to co - trimoxazole by malaria parasites; this may result in eventual resistance to sulphadoxine/ pyrimethamine.
4. There is no need for malaria prophylaxis in HIV patients on ART, since ARVDs have been known to have antimalaria properties

REFERENCES

Abdalla, S., Weatherall, D. J., Wickramasinghe, S. N., and Hughes, M. (1980). The anaemia of falciparum malaria. British Journal of Haematology 46 (2): 171-183

Abdalla, S. H., and Wickramasinghe S. N. (1988). A study of erythroid progenitor cells in the bone marrow of Gambia children with falciparum malaria. Clinical and Laboratory Haematology 10 (1): 33-40.

Adetifa, I. M. O., Akinsulie, A. O., Temiye, E. O., Iroha E. O., Ezeaka, V. G., Mafe, A. G. and Grange, A. O. (2008). Effect of Antiretroviral Therapy on Asymptomatic Malaria Parasiteamia in HIV-1 infected children. Nig. Postgrad. Med J 15: 120-125

Aidoo, M., Terlouw, D. J., Kolczak, M. S., McElroy, P. D., ter Kuile, F. O., Kariuki, S., Nahlen, B. L., Lal, A. A, and Uhayakumar, V. (2002). Protective effects of the sickle cell gene against malaria morbidity and mortality Lancet 359: 1311-1312

Aina, B. A., Tayo, F., Taylor, O. and Eniojukan, J. F. (2009). Antimalarial prescribing patterns in state hospitals and selected parastal hospitals in Lagos, Nigeria. Nig Qt. J. Hosp. Med. 19: 20-26

Alexander, T., Balaj, R. and Define L. (1994). Flow cytometric CD4 cell determinations based upon CD3 gating in HIV infected individuals. Cytometry 18**:**172.

Allison, A. C. (1954). The distribution of the sickle cell trait in East Africa and elsewhere and its apparent relationship with the incidence of sub tertian malaria. Trans R Soc Trop. Med. Hyg 48: 312-318

Allison, J. L., O'Brien, R. L. and Hahn, F. E. (1965) DNA: reaction with chloroquine. Science. 149: 1111-1113

Allison, J. L., O' Brien, R. L. and Hahn, F. E. (1966) Nature of the deoxyribonucleic acid-chloroquine complex. In Antimicrobial Agents and Chemotherapy – 1965. Sylvester S C (Ed) American Society for Microbiology. Ann Arbor, Michigan pp. 310-314.

Altman, L. K. (1981). Rare Cancer seen in 41 Homosexuals in *New York Times* Abe Rosenthal (Ed.).The New York Times Company, New York. p.1.

Alving, A. S., Eichelberger, L., Craige (Jr.), B., Jonesm (Jr.), R., Whorton, C. M., Pullman, T. N. (1948) Studies on the chronic toxicity of chloroquine (SN-7618) J. Clin. Invest 27: 60-65

Andrews, K. T., Fairlie, D. P., Madala, P. K. ,Ray, J., Wyatt, D.M., Hilton, P.M., Melville, L. A., Beattie, L., Gardiner, D.L., Reid, R .C., Stoermer, M.J.,

Skinner-Adams, T., Berry, C., and McCarthy, J. S., (2006) "Potencies of human immuno deficiency virus protease inhibitors *in vitro* against *Plasmodium falciparum* and *in vivo* against murine malaria," Antimicrobial Agents and Chemotherapy, 50(2): 639–648.

Anglaret, X., Chene, G., Attia, A., Toure, S., Lafont, S., Combe, P., Manlan, K., N'Dri-Yoman, T., and Salamon, R. (1999). Early chemoprophylaxis with trimethoprim-sulphamethoxazole for HIV-1-infected adults in Abidjan, Côte D'Ivoire: a randomized trial. Cotrimo-CI Study group. Lancet 353: 1463-1468.

Appay, V and Sauce, D., (2008). "Immune activation and inflammation in HIV-1 infection: causes and consequences," Journal of Pathology. 214, (2), 231–241.

Arndt, K.A., and Jick, H. (1976) Rates of cutaneous reactions to drugs. J.A.M.A. 235: 918-923.

Audu, R.A., Onwujekwe, D.I., Onubogu, C. C., Adedoyin,J .A., Onyejepu, N., Mafe, A. G., Onyewuche, J., Oparaugo, C., Enwuru, C., Aniedobe, M., Musa, A. Z., and Idigbe, E.O. (2005) Possible impact of co-infections of Tuberculosis and Malaria on the CD_4+ cells counts of HIV patients in Nigeria. Annals of African Medicine 4 (1): 10-13

Ayisi, G., van Eijk, A. M., Newman, R. D., ter Kuile, F. O., Shi, Y.P., Yang, C., Kolczak, M. S., Otieno, J. A., Misore, A. O., Kager, P. A., Lal, R. B., Steketee, R.

W. and Nahlen, B. L., (2004) "Maternal malaria and perinatal HIV transmission, Western Kenya," Emerging Infectious Diseases, . 10 (4): 643–652.

Ayouba, A., Badaut, C., Kfutwah, A., Cannou, C., Juillerat, A., Gangnard, S., Behr, C., Mercereau-Puijalon, O., Bentley, G., Barre-Sinoussi, F. and Menu, E., (2008.) "Specific stimulation of HIV-1 replication in human placental trophoblasts by an antigen of *Plasmodium falciparum*," AIDS, 229 (6): 785–787.

Ayouba, A., Nerrienet, E., Menu, E., Lobé, M.M., Thonnon, J., Leke, R.J.I., Barré-Sinoussi, F., Martin, P., and Cunin, P. FOR THE YAOUNDE MTCT GROUP (2003) "Mother-to-child transmission of human immunodeficiency virus type 1 in relation to the season in Yaounde, Cameroon," American Journal of Tropical Medicine and Hygiene, 69(4): 447–449.

Baird, J. K. (2000). Resurgent malaria at the millennium control strategies in crisis. Drugs 59: 719 – 743.

Ballin, J. C. (1974) Evaluation of a new topical agent for burn therapy. Silver sulfadiazole. J. A. M. A. 230: 1184 – 1185.

Balter, M. (1997). How does HIV overcome the body's T cell bodyguards? Science 278: 1399-1400.

B**altzan, M., Mehta, S., Kirkham, T. H. and Cosio, M. G. (1999).** Randomized trial of prolonged choroquine therapy in andvanced pulmonary sarcoidosis. Am. J. Respir. Crit Care Med. 160: 192-197.

Bamidele, O. (1997) Traditional therapy of asthma in Nigeria. Nig. Postgrad. Med. J. 4: 14-16.

Bastos, F., Barcellos, C., Lowndes, C. and Friedman, S. (1999) Co-infection with malaria and HIV in injecting drug users in Brazil: a new challenge to public health? Addiction 94:1165-1174.

Baum, M.K., Shor-Posner, G. and Lu, Y. (1995). Micronutrients and HIV-1 disease progression. AIDS 9: 1051-1056.

Berliner, R.W., Earle(Jr.), D. P.,Taggart, J.V., Zubrod, C. G., Welch, W. J., Conan, N. J., Bauman, E., Scudder, S.T., and Shannon, J. A.(1948) Studies on the chemotherapy of the human malarias VI..The physiological disposition, anti malarial activity, and toxicity of several derivaties of 4-aminoquinoline. J. Clin. Invest. 27:98 - 107

Bernstein, H. N., Svaifler, N. J., Rubin, M. and Mausour, A. M. (1963). The ocular deposition of chloroquine Invest. Ophhalmol. Visual Sci. 2: 384-392.

Birku, Y., Mekonnen, E., Bjorkman, A., and Wolday, D. (2002). Delayed clearance of *Plasmodium falciparum* in patients with human immunodeficiency virus co-infection treated with artemisinin. Ethiop Med J. 40(Suppl.1):17–26.

Bloland, P. B., Wirima, J. J., Steketee, R. W., Chilima, B., Hightower, A. and Breman, J. G. (1995). Maternal HIV infection and infant mortality in Malawi: evidence for increased mortality due to placental malaria infection**.** *AIDS*, 9:721-726.

Brooks, M. H., Malloy, J. P., Bartelloni, P. J., Sheehy, T. W. and Barry, K. G. (1969). Quinine, pyrimethamine and sulphurthodimethoxine: clinical response, plasma levels and urinary excretion during the initial attack of naturally acquired falciparium malaria. Clin. Pharmacol. Ther. 10: 85-91.

Brown, A. E., Webster, H. K., Teja-Isavadharm, P., and Keeratithakul, D. (1990) Macrophage activation in falciparum malaria as measured by neopterin and interferon- gamma. Clin Exp Immunol 82:97-101.

Brown, H., Rogerson, S., Taylor, T., Tembo, M., Mwenechanya, J., Molyneux, M. and Turner, G. (2000). "Blood-brain barrier function in cerebral malaria in Malawian children," American Journal of Tropical Medicine and Hygiene, 64 (3-4): 207–213.

Carteau, S., Mouscadet, J. F., Goulaouic, H., Subra,F. and Auclair, C. (1993). Inhibitory effect of the polyanionic drug Suramin on the *in vitro* HIV DNA integration reaction.Arch. Biochem, Biophys 305:606 – 610.

Center for Disease Control. (1984). Surveillance report.

Centre for Disease Control (1997). World malaria situation in 1994, part III Weekly Epidemiol. Rec. 72: 28 5-9.

Center for Disease Control and Prevention (2004). HIV and the disease AIDS update.

Chambers, H. F. (2001) Sulfonamides, Trimethoprime and quinolones In: Basic and Clinical Pharmacology. Katzung, B. G. (ed), 8th Edition. McGraw Hill Companies, Inc. New York. Pp. 793-802

Chandramohan, D., and Greenwood, B.M. (1998). Is there an interaction between human immunodeficiency virus and *Plasmodium falciparum*? Int J Epidemiol 27: 296-301.

Chau, T. T. H., Mai, N. T. H., Phu, N. H., Luxemburger, C., Chuong, L. V., Loc, P. P., Trang, T. T. M., Vinh, H., Cuong, B. M., Waller, D. J., Sinh, D. X. Day, N. P. J., Hien, T. T., and White, N. J. (2002). Malaria in injection drug abusers in Vietnam. Clin Infect Dis 34:1317-1322.

Chintu, C., Bhat, G. J., Walker, A. S, Mulenga V., Sinyinza, F., Lishimpi, K., Farrelly, L., Kaganson, N., Zumla, A., Gillespie, S., Nunn, A. J., and Gibb, D. M. (2004) Co-trimoxazole as prophylaxis against opportunistic infections in HIV-

infected Zambian children (CHAP): a double-blind randomised placebo-controlled trial. Lancet 364:1865–1871.

Chou, A. C., Chevli, R. and Fitch, C. (1980). Ferriprotoporphyrin IX fulfills the criteria for identification as the chloroquine receptor of malaria parasites. Biochemistry 19: 1543-1549.

Chun, T. W., Carruth, L., Finzi, D., Shen, X., Digiuseppe, J. A., Taylor, H., Hermankova, M., Chadwick, K., Margolick, J., Quinn, T. C., Kuo, Y. H., Brookmeyer, R., Zeiger, M.A., Barditch-Crovo, P. and Siliciano, R.F. (1997). Quantitification of latent tissue reservoirs and total body viral load in HIV-1 infection. Nature 387: 183-188.

Chun, T. W., Engel, D., Berrey, M. M., Shea T., Corey, L. and Fauci, A. S. (1998) Early establishment of a pool of latently infected, resting CD4 (+) T cells during primary HIV-1 infection. Proc. Natl. Acad. Sci. U.S.A., 95: 8869-8873

Clerici, M. and Shearer, G. M. (1993). "A TH1→TH2 switch is a critical step in the etiology of HIV infection," Trends in Immunology, 14 (3): 107–111.

Coatney, G. R., Cooper, W. C., Eddy, N. B. and Greenberg, J. (1953) Survey of Antimalarial Agents: Chemotherapy of *Plasmodium gallinaceum* Infections; Toxicity, correlation of structure and Action. Public Health Service Monograph No.9 U.S. Government Printing Office, Washington D.C, United States of America.

Cochrane Collaboration (2006). Insecticide-treated bed nets and curtains for preventing malaria Engelerc ed.In: The Cochrane Library, Issue 3. The Cochrane Collaboration. John Wiley and Sons Publishers Ltd Chichester, UK.

Cogswell, F. B. (1992). The hypnozoite and relapse in primate malaria clinical Microbiol. Rev. 5:26 -35.

Cohen, S. N. and Yielding, K. L. (1965). Inhibition of DNA and RNA polymerase reaction by choloroquine Proc. Natl Acad. Science USA 54: 521-527.

Colebunders, R., Bahwe, Y., Nekwei, W., Ryder, R., Perriens, J., Nsimba, K., Turner, A., Francis, H., Lebughe, I., and Van der Stuyft, P. (1990) Incidence of malaria and efficacy of oral quinine in patients recently infected with human immunodeficiency virus in Kinshasa, Zaire. J Infect; 21:167–73.

Corbett, E., Steketee, R., ter Kuile F., Latif A., Kamali, A., and Hayes, R. (2002) HIV-1/AIDS and the control of other infectious diseases in Africa *The Lancet*, 359(9324):2177-2187.

Craig, M. H., Kleinschmidt, I., le Sueur, D. and Sharp, B. L. (2004). Exploring thirty years of malaria case data in KwaZulu-Natal, South Africa, Part II: the impact of non-climatic factors. Trop Med Int Health 9:1258–1266.

Craig, M.H., Snow, R.W., and le Sueur, D. (1999). A climate-based distribution model of malaria transmission in sub-Saharan Africa. Parasitol Today.15:105–111.

Danning, C. L. and Boumpas, D. T. (1998). Commonly used disease-modifying antirheumatic drugs in the treatment of inflammatory arthritis: an update on mechanisms of action. Clin. Exp. Rheumatol 16: 595-604

Davidson, M. W., Griggs (Jr.), B. G., Boykin, D. W. and Wilson, W. D. (1977). Molecular structural effects involve in the interaction of quinoline methanolamines with DNA. Implication for antimalarial action. J. Med. Chem. 20: 1117-1122.

Davis, T. M., Skinner-Adams, T. S., and Beilbyb, J. (1998) *In vitro* growth inhibition of *Plasmodium falciparum* by retinol a concentrations present in normal human serum Acta Tropica 69: 111-119.

Dayachi, F., Kabongo, L. and Ngoie, K. (1991) Decreased mortality from malaria in children with symptomatic HIV infection. Int. Conf AIDS 16-21 Jun; 2: 164.

Deeks, S. G. and Volberding, P.A. (1999). Antiretroviral Therapy In: The Medical Management of AIDS.Sande, M. A., Volberding P.A. (eds) W.B.Sunders Company, 6th edition.Philadelphia. pp. 97-115.

Dembo, G., Mwapasa, V., Montgomery, J. , **Craig, A.G., Porter, K.A., Meshnick, S. R., Molyneux, M. E. and Rogerson, S. J. (2008).** "Impact of human immunodeficiency virus infection in pregnant women on variant-specific immunity to malaria," Clinical and Vaccine Immunology, 15 (4) 617-621.

Department of Health and Human Services (2004). A guide to Primary Care for people with HIV/AIDS, 2004 edition DHHS, Washington D C, USA.

Department of Health and Human Services (2005). Guidelines for the use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents October edition HH, Washington, USA.

Department of Health and Human Services (2006). HIV and its treatment: What You Should Know. August edition. DHHS, Washington, USA.

Donnol, L., Sangiuneti, V., Ricciardi, M. L. and Sodati, M. (1969) Anti malaria activity of kelfizina-pyrimethamine versus chloroquine in field trial in Nigeria. Am J Trop Med. Hyg 18: 182-187.

Drabkin, D. L. and Austin, J. H. (1935). Spectrophotometric Studies. II...Preparations from washed blood cells; nitric oxides hemoglobin and sulfhemoglobin J Biol Chem 112:51 – 65.

Dubois, E. L. (1978). Anti malarials in the management of discoid and systemic lupus erythematosus. Semin. Arthritis Rheum. 8: 33-51.

Dutta, P. and Fitch, C. D. (1983). Diverse membrane-active agents modify hemolyic response to ferriproporphyrin IX. J. Pharmacol. Exp. Ther. 225: 729-734.

Dybul, M., Fauci, A. S., Barlett, J. G., Kaplan, J. E. and Pau, A. K. (2002). Guidelines for using antiretroviral agents among HIV-infected adults and adolescents Ann. Intern med 137 (5Pt 2): 381-433.

Easterbrook, M. (1999). Deteection and prevetion of maculopathy associated with antimalaria agents. Int. Ophtalmol. Clin. 39: 49-57.

Falco, E. A., Goodwin, L. G., Hitchings, G. H., Rollo, I. M. and Russell, P. B. (1951) 2: 4 Diaminopyrimidines-a new series of antimalarials. Br. J. Pharmacol Chemother., 6:185-200.

Federal Ministry of Health (2000). Malaria control in Africa in the new millennium, a working document prepared for the African summit on Roll Back Malaria initiative, 1 – 69.

Federal Ministry of Health (2008). Standard treatment guidelines**.** FMOH/WHO/EC/DFID, Abuja,1st edition.

Ferone, R., Burchatt, J. J. and Hitchings, G. H. (1969) *Plasmodium berghei* dihydrofolate reductase: isolation, properties inhibition by antifolates. Mol. Pharmacol. 5: 49-59.

Fesen, M. R., Kohn, K. W., Leteurtre, F. and Pommier, Y.(1993). Inhibitors of human immunodeficiency virus integrase. Proc. Natl Acad, Sci. USA 90: 2399 – 2403.

Finzi, D., Hermankova, M., Pierson, T., Carruth, L.M., Buck, C., Chaisson, R. E., Quinn, T. C., Chadwick, K., Margolick, J., Brookmeyer, R., Gallant, J., Markowitz, M., Ho, D. D., Richman, D. D. and Siliciano, R. F. (1997). Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. Science 278: 1295-1300.

Fitch, C. D. (1983) Mode of action of anti malarial drugs. In: Malaria and the Red cell. Ciba Foundation Symposium 94. Pitman, London. Pp. 222-232.

Flexner, C. (2000). Dual protease inhibitor therapy in HIV-infected patients: pharmacologic rationale and clinical benefits. Annu Rev. Pharmacol.Toxicol. 40: 649-674.

Food and Drug Administration (2005). FDA Approved Drugs for HIV/AIDS and Related conditions. pp. 1-6.

French, N., and Gilks, C.F. (2000) Fresh from the field: some controversies in tropical medicine and hygiene: HIV and malaria, do they interact? Trans R Soc Trop Med Hyg 94: 233-237.

French, N., Nakiyingi, J., Lugada, E., Watera, C., Whitworth, J. and Gilks, C. (2001). Increasing rates of malarial fever with deteriorating immune status in HIV-1 infected Ugandan adults. AIDS 15: 899-906.

Friedman, M. J. (1978). Erthrocytic mechanism of sickle cell resitance to malaria Proc Nalt Acad Sci USA 75 (4): 1994-1997.

Fritsch, C., Lang, K., von Schmiedeberg, S., Bolsen, K., Merk, H., Lehmann, P. and Ruzicka, T. (1998) Porphyria cutanea tarda. Skin. Pharmacol. Appl. Skin Physiol. 11: 321-335

Froebel, J., Howard, W., Schafer, J. R., Howie, F., Whitworth, J. and Kaleebu, P. (2004). Activation by malaria antigens renders mononuclear cells susceptible to HIV infection and re-activates replication of endogenous HIV in cells from HIV-infected adults. Parasite Immunol: 26:213-217.

Garhnham, P. C. C. (1996). Malaria Parasites another haemosporidia. Blackwell Scientific Publications, oxford p. 1114.

Gendelman, H. and Grant, E. (2005). The Neurology of AIDS. Everall I. P. and Lipton S. A. (eds).Oxford University Press, London.

Geleziunas, R. and Greene, W.C. (1999). Molecular insights into HIV- 1 infection and Pathogenesis.In: The Medical Management of AIDS.Sande, M. A., Volberding P.A.(eds) W.B.Sunders Company , .Philadelphia 6th edition. pp.23-39.

Gilles, H. M. (2002). Historical outline: milestones in the history of malaria and its control. In: Essential malariology. Warrell, D. A., Gilles, H.M. (eds). 4th edition. Arnold, London pp.1-7.

Good, M. F., and Doolan, D. L. (1999). Immune effector mechanisms in malaria. Curr Opin Immunol 11:412-419 .

Good M. I. and Shader R. I. (1982). Lethality and behavioural side effects of chloroquine J. Clin. Psychopharmacol 2: 40-47.

Goodman, C. A., Coleman, P. G. and Mills, A. J. (1999). Cost-effectiveness of malaria control in sub-Saharan Africa. Lancet 354 (9176): 378-385.

Graham, C.S., and Koziel, M. J. (2000). Prophylactic effect of malarone against malaria: all good news. Lancet 356: 1864-1860.

Graziosi, C., Soudeyns, H., Rizzardi, G. P., Bart, P. A., Chapuis, A. and Pantaleo, G. (1998). Immunopathogenesis of HIV infection. AIDS Res Hum Retroviruses; 14: S 135-S 142 .

Greenspan, H. C., and Aruoma, O. I. (1994) Oxidative stress and apoptosis in HIV infection: a role for plant derived metabolites with synergistic antioxidant activity. Immuno Today 15: 209-213.

Greenwood, D. (2000). , Protozoa: Malaria, Toxoplasmosis, Cryptosporidiosis, Amoebic dysentery, Sleeping sickness, Chagas' disease, Leishmaniasis, Giadiasis, Trichomoniasis, Pneumocystis pneumonia. In: Medical Microbiology, A guide to Microbial infections: Pathogenesis, Immunity, Laboratory diagnosis and control. Greenwood D., Slack R., Peutherer J. (eds).Harcourt Publishers Limited ,London, 15th edition, Pp. 577-586.

.

Griffin, G. E., Sissons, J. G. P., Chiodini, P. L., Mitchell, D. M. (1999). Diseases due to infection. In: Davidson's principles and Practice of Medicine. Haslett C., Chilvers E.R,, Hunter J.A.A., Boon N.A. (eds), Harcourt Publishers,Limited, London, 18th edition, pp. 57-190

Hamel, M. J., Odhacha, A, Roberts, J. M., Deming, M. S. (2001) Malaria control in Bungoma, Kenya: a survey of home treatment of children with fever, bednet use and attendance at antenatal clinics. Bulletin of the World Health Organization 79 (11):1005 -1092.

Hammer, S. M., Eron, J. J. Jr., Reiss, P., Schooley, S., Cahn, P., Fischl, M.A., Gatell, J. M., Hirsch, M. S., Jacobsen, D. M., Montaner, J. S .G., Richman, D. D., Yeni, P. G. and Volberding, P. A. (2008). Antiretroviral Treatment of Adults HIV Infection: 2008 Recommendations of the International AIDS Society USA Panel JAMA 300 (5): 555-570.

Hamzah, J., Davis, T. M. E., Skinner-Adams, T. S. and Beilbyb, J. (2004). Characterization of the effect of retinol on *Plasmodium falciparum* in vitro Experimental Parasitology 107: 136-144.

Harris, K. S., Brabant, W., Styrchak, S., Gall, A. and Daifuku, R. (2005). KP – 1212/1461, a nucleoside designed for the treatment of HIV by viral mutagenesis. Antiviral Research 67: 1 – 9.

Harrison, H. N., Bales, H. W. and Jacob, F. J. (1972). The absorption into burned skin of sulfamylon acetate from 5 percent aqueous solution. J. Trauma 12: 994 – 998.

Harrison, N. E., Ijoma, C. K., Odunukwe, N. N. and Agomo, C. O. (2004). Malaria treatment in Enugu Urban Nigeria: Physiciancs' compliance with the National treatment Guideleines. Nig. Qt. J. Hosp.Med. 14: 227-232

Hebert, P. D. N., and Beaton, M. J. (1993). Methodologies for Allozyme Analysis Using Cellulose Acetate Electrophoresis: A Practical Handbook. Helena Laboratories (technical manual), Beaumont, Texas p. 32.

Hetzel, M. W., Iteba, N., Makemba, A., Mshana, C., Lengeler, C., Obrist, B., Schulze, A., **Nathan, R., Dillip, A., Alba, S., Mayumana, I., Khatib, R. A., Njau, J**. **D.,and Mashinda, H. (2007)** Understanding and improving access to prompt and effective malaria treatment and care in rural Tanzania: the access Programme. Malaria Journal 6: 83.

Hewitt, K., Steketee, R., Mwapasa, V., Whitworth, J. and French, N. (2006). Interactions between HIV and malaria in non-pregnant adults: evidence and implications AIDS 20(16): 1993-2004.

Hien, T. T.and White, N. J. (1993). Oinghaosu. Lancet 341: 603-608.

Hill, A. V., Allsopp, C. E., Kwiatkowski, D., Anstey, N. M., Twumasi, P., Rowe, P. A., Bennett, S., Brewster, D., McMichael, A. J. and Greenwood, B. M. (1991). Commonest West African HLA antigens are associated with protection from severe malaria Nature, 352: 595-600.

Hirsch, M.S., Gunthard, H. F., Schapiro, J. M., Brun-Vézinet, F., Clotet, B., Hammer, S. M., Johson, V. A., Kuritzkes, D. R., Mellors, J. W. and Pillay, D. (2008). Antiretroviral drug resistance testing in adult HIV-1-infection 2008 recommendations of an international AIDS Society - USA Panel. Clin Infect Dis 47: 266-285.

Hitchings, G. H. and Burchall, J. J. (1965). Inhibition of folate biosynthesis and function as a basis for chemotherapy. Adv. Enzymol 27: 417-468.

Hobbs, C. V., Voza, T., Coppi, A., Kirmse, B., Marsh, K., Borkowsky, W. and Sinnis, P. (2009). "HIV protease inhibitors inhibit the development of preerythrocytic-stage Plasmodium parasites," Journal of Infectious Diseases, 199(1): 134–141.

Hochman, S. and Kim, K. (2009). The impact of HIV and Malaria co-infection: What is known and suggested venues for further study. Interdisciplinary Perspectives on Infectious Diseases. Tanowitz H. B., (ed.) Vol 10.1155.Hindawi Publishing Corporation, New York.

Hudson, A. T., Dickins, M., Ginger, C. D., Gutteridge, W. E., Holdich, T., Hutchinson, D. B., Pudney, M., Randall, A. W. and Latter, V. S. (1991). A potent broad spectrum anti-infective agent with activity against malaria and opportunistic infections in AIDS patients' Drugs Exp. Clin. Res. 17: 427-435.

Hughes, W. T., Gray, V.L., Gutteridge, W. E., Latter, V.S. and Pudney, M. (1990). Efficacy of hydroxyl naphtha quinones in experimental pneumocystis carini puemonitis. Antimicrob. Agents Chemother 34: 225-228.

Hurly, M. G. D. (1995). Potentiation of primethamine by sulphadiazine in human malaria. Trans. R. Soc. Trop. Med. Hyg 53: 412-413.

Isaacson, D., Elgart, M., Turner, M. L. (1982). Antimalarials in dermatology Int. J. Dermatol 21: 379-395.

Iyer, J. K., Milhous, W. K, Ccortese, J. F., Kublin, J. G. and Plowe, C. V. (2001). *Plasmodium falciparium* cross-resistance between trimethoprim and pyrimthamine. Lancet 358: 1066-1067.

Jones, G., Stekee, R.W., Black, R. E., Bhitta, Z. A., and Morris, S. S. (2003). How many child deaths can we prevent this year? Lancet 362:65 -71.

Kager, P. A., Schultz, J., Zijlstra, E. E. and Van Boxtel, C. J. (1994). Arteether administration in humans: preliminary studies of pharmacokinetics, safety and tolerance. Trans. R. Soc. Trop. Med. Hyg. 88: 53-54.

Kalowski, S., Nanra, R. S, Mathew, T. H. and Kincaid-Smith, P. (1973). Deterioration in renal function in association with co-trimoxazole therapy. Lancet 2: 394-397.

Kamya, M. R., Kigonya, C. N. and McFarland, W. (2001) HIV infection may adversely affect clinical response to chloroquine therapy for uncomplicated malaria in children. AIDS 15: 1187 - 8.

Kates, J., Sorian, R., Crowley, J. S. and Summers, T. A. (2002). Critical Policy Challenges in the Third Decade of the HIV/AIDS Epidemic**.** Am. J. Public Health 92: 1060 -1063.

Keen, J., Serghides, L., Ayi, K., Patel, S. N., Ayisi, J., van Eijk, A., Steketee, R., Udhayakumar, V. and Kain, K. C. (2007). "HIV impairs opsonic phagocytic clearance of pregnancy-associated malaria parasites," PLoS Medicine 4(5), article e181.

Keschamrus, N., Noeypatimanond, S., Onrumpun, P. and Sarikabhuti, B. (1988). Urinary level of sulphadoxine e in falciparium malaria patients after treatment with sulphadoxine -pyrimethamine. Journal of the Medical Association of Thailand 71:33 - 39.

Khasnis, A. A., and Karnad, D. (2003). Human immunodeficiency virus type 1 infection in patients with severe falciparum malaria in urban India. J Postgrad Med 49:114-117.

Knapp, D. A., Knapp, D. E., and Engel, J. F. (1981). The Public, the pharmacists and self-medication In-pharmacy practice, social and behavioural aspect .Werthemer A I and Smith M eds. 2nd ed. Baltimore: University Park Press: 99.

Korenromp, E. L.,Williams, B. G., de Vlas, S. J., Gouws, E., Gilksn, C. F., Ghys, P. D. and Nahlen, B. L. (2005). Malaria attributable to the HIV-1 epidemic, sub-Saharan Africa.Emerg Infect Dis; 11:1410 – 1419.

Kowalska, J. D., Mocroft, A., Blaxhult, A., Colebunders, R., Lunzen, J., van Podlekareva, D., Hansen, A. E., Machala, L., Yust, I. and Benfield, T. (2007). Current Hemoglobin Levels are more predictive of disease progression than Hemoglobin measured at baseline in patients receiving Antiretroviral treatment for HIV Type 1 Infection**.** AIDS Research and Human Retroviruses. 23 (10): 1183-1188.

Kublin, J., Jere, C., Miller. W., Hoffman, N., Chimbiya, N. and Pendame, R. (2005). Effect of *Plasmodium falciparum* malaria on concentration of HIV-1-RNA in the blood of adults in rural Malawi: a prospective cohort study. Lancet; 365:233-240**.**

Kuritzkes, D. R. (2004) Diagnostic tests for HIV infection and resistance assays.In: Infectious Diseases.Cohen J. et al., (eds).Mosby, Spain,2nd edition pp. 1369-1378.

Landsteiner, K., and Levine, P. (1927) .A new agglutinable factor differentiating individual human bloods.Proc. Soc. Exp. Biol NY 24: 600 – 602.

Lasagna, (1970). Calls for more self-medication.American Druggist 162: 31.

Levy, J. A. (2006). HIV Pathogenesis: Knowledge gained after two decades of Research. Adv. Dent. Res. 19: 10 – 16.

Lucas, A. O., Hendrickse, R.G., Okubadejo, O. A., Richards, W. H. G., Neal, R. A. and Kofie, B. A. K. (1969). The suppression of malarial parasitaemia by pyrimethamine in combination with dapsone or sulphormethoxine. Trans R. Soc. Trop. Med. Hyg 63: 216-229.

Makani, J. M., Atuja, W., Liyombo, E., Snow, R. W., Marsh, K. and Warrell, D. A. (2003). Admission diagnosis of cerebral malaria in adults in an endemic area of Tanzania: implications and clinical description. Qt. J. Med 96: 355-362.

Mandell, G. L., and Sande, M. A. (1980). Antimicrobial Agents: Sulfonamides,Trimethoprim-Sulfamethoxazole,and urinary tract antiseptics. In: The Pharmacological Basis of Therapeutics. Gilman A.G., Goodman L. S., and Gilman A. (eds). Macmillan Publishing Co., Inc., New York, 6th edition, pp. 1106-1125.

Mandell, G. L., and Sande, M. A. (2001). Antimicrobial Agents: Sulfonamides,Trimethoprim-Sulfamethoxazole,and urinary tract antiseptics. In: The Pharmacological Basis of Therapeutics. Gilman A.G., Goodman L. S., and Gilman A. (eds). Harcourt Publishing Co., New York, 11th edition, pp. 1112-1146.

Marsh, K., Otoo, L, Hayes, R. J., Carson, D. C. and Greenwood, B. M. (1989). Antibodies to blood stage and antigens of plasmodium falciparum in rural Gambians and their relations to protection against infection Trans R Soc. Trop. Med Hyg 83: 293-303.

Masika, P. M., Semarndu, W. J., Urassa, R., Mosha, J., Chandramohan, D., and Gosling, R. D. (2006). Over diagnosis of malaria is not a lost cause. Malaria Journal 5: 120.

Menendez, C., D'Alessandro, U., ter Kuile, F.O. (2007). Reducing the burden of malaria in pregnancy by preventive strategies. Lancet Infect.Dis. 7(2):126-135.

Menendez, C., Fleming, A. F., and Alonso, P. L. (2000). malaria-related anemia. Parasitology Today 16 (II): 469-476.

Merchant, R. C., Mayer, K. H., and Browning, C .A. (2002). Non occupational HIV post-exposure prophylaxis: guidelines for Rhodes Island from the Brown University AIDS Programme and the R I Department of Health Med. Health RI 85: 244-248.

Mermin, J., Lule, J., Ekwaru, J. P., Malamba, S., Downing, R., Ransome, R., Kaharuza, F., Culver, D., kizito, F., Bunnell, R., Kigozi, A., Nakanjako, D., Wafula, W. and Quick, R. (2004). Effect of Co-trimoxazole prophylaxis on morbidity, mortality, CD_4 count and viral load. Lancet 364: 1428-1434.

Mermin, J., Lule, J., Ekwaru, J. P., Downing, R., Hughes, P., Bunnel, R., Malamba, S., Ransome, R., kaharuzza, F., Coutinho, A., Kigozi, A., Quick, R. (2005). Co-trimoxazole prophylaxis by HIV-infected persons in Uganda reduces morbidity and mortality among HIV-infected family members. AIDS 10: 1035-1042.

Mermin, J., Ekwaru, J. P., Liechty, C. A., Were, W., Downing, R., Ransom, R., Weidle, P., Lule, J., Coutinho, A. and Solberg, P.(2006). Effect of co-trimoxazole prophylaxis, antiretroviral therapy and insecticide-treated bednets on the frequency of malaria in HIV-1 infected adults in Uganda: a prospective cohort study Lancet 367 (9518) 1256-1261.

Meshnick, S. R. (1994). The mode of action of antimalarial endoperoxides. Transactions of the Society of Tropical Medicine and Hygiene 88, Suppl. 1: 31-32.

Meshnick, S. R. (2002). Artemisinin: mechanisms of action, resistance and toxicity. Int. J. Parasitol. 32: 1655-1660.

Meshnick, S. R., Chang, K. P. and Cerami, A. (1977). Hemolysis of the blood stream forms of *Trypanosoma brucei*. Biochem. Pharmacol 26: 1923-1928.

Meshnick, S. R., Yang, Y. Z., Lima, V., Kuypers, F., Kamchonwongpaisan, S. and Yuthavong, Y. (1993). Iron-dependent free radical generation from the antimalarial agent artemisinin (qinghaosu). Antimicrob Agents Chemother 37:1108-1114.

Mohan, K., Dubey, M. L., Ganguly, N. K. and Mahajan, R. C. (1995). *Plasmodium falciparum*: role of activitated blood monocytes in erythrocyte membrane damage and red cell loss during malaria. Experimental Parasitology 80(I): 54-63.

Moodley, D., Moodley, J. and Coovadia, H. (2003). A multicenter randomize controlled trial of nevirapine versus a combination of zidovudine and lamivudine to reduce intrapartum and early postpartum mother-to-child transmission of human immuno deficiency virus type I J Infect Dis 187: 725-735.

Moore, J. M., Ayisi, J., Nahlen, B. L., Misore, A, Lal, A. A. and Udhayakumar, V. (2002). Immunity, to placental malaria II Placental antigen-specific cytokine responses, is impaired in human immunodeficiency virus-infected women. J Infect Dis182: 960-964.

Muentener, P., Shlagenhauf, P. and Steffen, R. (1999). Imported malaria between 1985 and 1995: trends and perspectives. Bull. World Health Organization 77: 560-566.

Muller, O. and Moser, R. (1990). The clinical and parasitological presentation of *Plasmodium falciparum* malaria in Uganda is unaffected by HIV-1 infection**.** Trans R Soc Trop Med Hyg, 84:336-338.

Mullis, K. B. and Faloona, F. A. (1987). Specific synthesis of DNA *in vitro* via a polymerase-catalyzed chain reaction. Methods in Enzymology 155:335 – 350.

Murphy, S. C. and Breman, J. G. (2001). Gaps in childhood malaria burden in Africa cerebral malaria, neurological sequelae anemia respiratory distress, hypoglycaemia and complications of pregnancy.Am J. Trop. Med Hyg. 64, Suppl. 57 – 67.

Mwapasa, V., Rogerson, S. J., Molyneux, M. E., Abrams, E. T., Kamwendo, D. D., Lema, V. M., Tadesse, E., Chaluluka, E., Wilson, P. E. and Meshnick, S. R. (2004). The effect of *Plasmodium falciparum* malaria on peripheral and placental HIV-1 RNA concentrations in pregnant Malawian women. Aids 18:1051- 1059.

Nathoo, S., Serghides, L. and Kain, K. C. (2003). "Effect of HIV-1 antiretroviral drugs on cytoadherence and phagocytic clearance of *Plasmodium falciparum*-parasitised erythrocytes," The Lancet 362(9389): 1039–1041.

National Action Committee on AIDS (2006). HIV/AIDS Statistics. Federal Ministry of Health, Abuja.

Nicholson, J. K. A., Hubbard, M. and Jones. B. M. (1996). Use of CD45 fluorescence and side scatter characteristics for gating lymphocytes when using the whole blood lysis procedure and flow cytometry. Cytometry 26:16-21

Nielson, N. O., Simonsen, P. E., Magnussen, P., Magesa, S. and Friss, H. (2006). Cross-sectional relationship between HIV, lymphatic filariasis and other parasitic infections in adults in coastal northeastern Tanzania. Trans R Soc Trop Med Hyg. 100: 543–550.

Okafor, I. P., and Odeyemi, K. A. (2009) Home management of malaria by care givers of under-five children in an urban area in Lagos, Nigeria. Nig. Qt J. Hosp. Med. 19: 37-41

Olansky, A. J. (1982). Antimalarial and ophthalmologic safety. J. Am Acad. Dermatol. 6: 19-23.

Olayemi, S. O., Oreagba, I. A. and Mabadeje, A. F. B. (2004). Knowledge and practice of the use of Insecticide- Treated Nets among Mothers in Lagos, Nigeria. (A prelimianary Survey). Nig. Qt. J. Hosp. Med. 14: 181-184.

Oshinaike, O. O., Okubadejo, N. U., Ojini, F. I. and Danesi, M. A. (2009). The Clinical Spectrum of Neurological Manifestations in HIV patients on HAART at the Lagos University Teaching Hospital, Lagos, Niegeria. Nig. Qt. J.Hosp. Med. 19: 181-185.

Otubanjo, O. A., Mafe, M. A., Idowu, E. T and Adeneye, A. K. (2000). Knowledge, attitude and perception of malaria in Lagos State.Nig. Qt. J. Hosp. Med. 10 :73 – 77.

Parikh, S., Gut, J., Istvan, E., Goldberg, D. E., Havlir, D. V. and Rosenthal, P. J. (2005). "Antimalarial activity of human immunodeficiency virus type 1 protease inhibitors," Antimicrobial Agents and Chemotherapy 49 (7): 2983-2985.

Parise,M. E., Ayisi, J. G., Nahlen, B. L., Schultz, L. J.,Roberts, J. M., Misore, A., Muga, R., Oloo, A. J. and Steketee, R. W.(1998). Efficacy of sulfadoxine – pyrimethamine for prevention of placental malaria in an area of Kenya with a high prevalence of malaria and human immunodeficiency virus infection. Am. J. Trop. Med. Hyg. 59:813 – 822.

Pennisi, E. (2001). Malaria's beginnings on the heels of hoes? Science 293:416 – 417.

Perelson, A. S., Neumann, A. U., Markowitz, M., Leonard, J. M. and Ho, D. D. (1996). HIV-1-dynamics in vivo: virion clearance rate, infected cell life-spam and viral generation time Science 271 (5255): 1582-1586.

Perrault, D., Hajek, J. and Zhong, K. (2009). "Human immunodeficiency virus co-infection increases placental parasite density and transplacental malaria transmission in western Kenya," American Journal of Tropical Medicine and Hygiene 80(1):119–125.

Peters, W. (1965) Drug resistance in *Plasmodium berghei* Vinke and Lips: I Chloroquine resistance Expt Parasitol 17:80-89.

Petri, W. A. (2001). Sulphonamides, Trimethoprime, Sulfamethoxazole, Quinolones and Agents for Uninary tract infections. In: Goodman and Gilman's The Pharmacological Basis of Therapeutics. Hardman J. G., Limbirds L., Gilman A. G., (eds.) McGrams Hill Medical Publishing Division,New York, 10th edition: 1171-1188.

Petri, W. A. (2006). Sulphonamides, Thrimethoprime. Sulfamethoxazole, Quinolones and Agents for Uninary tract infections. In: Goodman and Gilman's The Pharmacological Basis of Therapeutics. Brunton L. L., Lazo J. S., Parker K. L. (eds). McGraw-Hill, New York. 11th edition, 1111-1126,

Peutherer, J. F. (2000). Retroviruses: Leukemia, lymphoma, acquired immune deficiency syndrome. In: Medical microbiology, A guide to microbial infections: Pathognesis, immunity, Laboraratory Diagnosis and control. Greenwood D., Slack R., Peutherer J . (eds) Harcourt Publishers Limited , London, 15th edition pp 518-27

Phillips, R. S. (2001) Current status of malaria and potential for control. Clinical Microbiology Reviews 14**:**208 -226.

Poveda, E., Briz, V. and Soriano, V. (2005). Enfuvirtide , the first fusion inhibitor to treat HIV infection. AIDS Rev. 7 : 139 – 147.

Prakash, D., Fesel, C., Jain, R., Cazenave, P. A., Mishra, G.C. and Pied, S. (2006). "Clusters of cytokines determine malaria severity in *Plasmodium falciparum*-infected patients from endemic areas of central India," Journal of Infectious Diseases 194(2):198–207.

Raffanti, S. and Haas, D. W. (2001). Antimicrobial agents: Antiretroviral agents. In Goodman and Gilman's The Pharmacological basis of Therapeutics. Hardman J. G., Limbirds L., Gilman A. G., (eds.) McGrams Hill Medical Publishing Division , New York,10th edition, pp.1349-1380.

Rang, H. P., Dale, M. M., Ritter, J. M., and Flower, R. J.(2008) Absorption and Distribution of Drugs In:Rang and Dale's Pharmacology.McGrath S.,Cook L.(eds).Churchill Livingstone Elsevier, China, 6th Edition, pp. 99 -112

Rang, H. P., Dale, M. M., Ritter, J. M., and Flower, R. J.(2008) Drug Elimination and Pharmacokinetics In:Rang and Dale's Pharmacology.McGrath S.,Cook L.(eds).Churchill Livingstone Elsevier, China, 6th Edition, pp. 113 -127

Rathbun, R. C. (2000). Parasitic Infections: Malaria. In: Textbook of Therapeutics, Drug and Disease Management. Herfindel E. T., Gourley D. R., Bergestroom K. A., Helms R. A., Kapusnik-Ujner J. E., Lynch S., Quan D. J., Rawls N., Self T. H. (eds) Lippincott Williams and Wilkins,Baltimore, 7th edition,pp. 1631-1634.

Reibnegger, G., Boonpucknavig, V., Fuchs, D., Hausen, A., Schmutzhard, E. and Wachter, H. (1984). Urinary neopterin is elevated in patients with malaria. Trans Roy Soc Trop Med Hyg 78:545-546.

Rihet, P., Flori, L., Tall, F., Traore, A. S. and Fumoux, F. (2004). Hemoglobin C is associated with reduced Plasmodium falciparum parasitaemia and low risk of mild malaria attack. Human Molecular genetics 13 (1): 1-6.

Roesenkranz, H. S., and Rosenkranz, S. (1972). Silver Sulfadiazine: interation with isolate deoxyribonucleic acid. Antimicrob. Agents Chemother., 2: 373-383.

Rollo, I. M. (1955). The mode of action of sulphonomides, proguanil and pyrimethamine on *Plasmodium gallinaceum*. Br. J. Pharmacol. Chemother 10: 208-214.

Rollo, I. M. (1980). Drugs used in the chemotherapy of malaria. In: The Pharmacological Basis of Therapeutics. Gilman A.G., Goodman L. S., and Gilman A. (eds). Macmillan Publishing Co., Inc., New York, 6th edition, pp. 1038-1060.

Roper, C., Pearse, R., Bredenkamp, B., Gumede, J., Drakley, C., Mosha, F., Chandramohan, D. and Sharp, B. (2003). Antifolate anti-malaria resistance in Southeast Africa: a population based analysis. Lancet 361:1174 – 1181.

Rozendaal, J. A. (1989). Impregnated mosquito nets and curtains for self-protection and vector control Tropical diseases Bulletin 86: R1-R41.

Rozendaal, J. A., Voorham, J., Van Hoof, J .P. and Oost burg, B. F. (1989). Efficacy of mosquito nets treated with permethrine insuriname.Medical and Vertinary Entomology 3(4): 353-365.

Russel R. B., Falcon, E. A., Boodwin, C. G., Hitchings, G. H., and Rollo, I. M. (1951). 2, 4 Diaminopyridines new series of antimalarias.Br J. Pharmac. 6**:**185-200.

Russell, S. (2003). The economic burden of illness for households: A review of cost of illness and coping strategy studies focusing on Malaria, Tuberculosis and HIV/AIDS. DCPP Workshop. Johannesburg, Disease Control Priority Project, Working Paper 15.

Ryley, J. F. and Peters, W. (1970). The antimalaria activity of some quinolone esters. Am Trop Med Parasitol 84:209-222.

Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B. and Erlick H.A. (1988). Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase.Science 239:487 – 491.

Samba, E. (2001). The malaria burden and Africa. Am J Trop Med Hyg. 64, suppl. 1 – 2.

Savarino, A., Lucia, M. B., Rastrelli, E., Rutella, S., Golotta, C., Morra, E., Tamburrini, E., Perno, C. F., Boelaert, J. R., Sperber, K., and Cauda, R. M. (2004). "Anti-HIV effects of chloroquine: inhibition of viral particle glycosylation and synergism with protease inhibitors," Journal of Acquired Immune Deficiency Syndromes, 35 (3): 223–232.

Schellengberg, K. A. and Coatney, G. R. (1960). The influence of antimalarial drugs on nucleic acid synthesis *in Plasmoium gallinaceum* and *Plasmodium berghei*. Biochem. Pharmaco. l 6: 143-152.

Schoepflim, S., Marfurt, J., Goroti, M., Baisor, M., Mueller, I. and Felger, I. (2008). Heterogeneous distribution of pf drug resistance haplotypes in Subsets of the host population. Malar. J .7: 78.

Scholte, E-J., Ng'habik, Kihonda, J., , W., Paaijman, S. K., Abdulla, S, Killeen G. F., and Knols, B. G. J.(2005): An Entomopathogenic fungus for control of adult African malaria mosquitoes. Science 308 : 1641 – 1642.

Serghides, L, and Kain, K. C. (2002) Mechanism of protection induced by vitamin A in falciprum malaria Lancet 359: 1404-1406.

Seyler, C., Anglaret, X., Dakoury-Dogbo, N., Messou, E., Toure, S., Danel, C., Diakite, N., Daudie, A., Inwoley, A., Maurice, C., Tonwe-Gold, B., Rouet, F., N'Dri-Yoman, T. and Salamon, R. (2003) Medium-term survival, morbidity and immunovirological evolution in HIV-infected adults receiving antiretroviral therapy, Abidjan, Cote d'Ivoire. Antivir Ther.8:385–93.

Shankar, A. H., Genton, B., Semba, R .D., Baiser, M., Paino, J., Tamja, S., Adiguma, T., Wu, L., Rare, L., Tielsh, J.M., Alpers, M .P.,and West, K. P. (1999) Effect of vitamin A supplementation on morbidity due to *Plasmodium falciparum* in young children in Papua New Guinea: a randomized trial Lancet 354: 203-209.

Sharp, B., van Wyk, P., Sikasote, J.B., Banda, P., and Kleinschmidt, I. (2002) Malaria control by residual insecticide spraying in Chingola and Chililabombwe, Copperbelt Province, Zambia. Trop Med Int Health7:732–6.

Shida, K. K., Lewchalermvongse, B. and Pang, L. W. (1989) *Plasmodium berghei* malaria infection causes increased cardiac output in rats, Rattus rattus Exp Parasitol 68:253 – 259.

Short, H. E., Fairley, N. H., Govell, G., Shue, P. G., and Garnham, P .C. C. (1949). The pre-erythrocytic stage of plasmodium. A preliminary note. Brit Med. Jour. 2: 1006-1008.

Short, H. E., Fairley, N. H., Govell, G., Shute, P. G. and Garnham, P. C. C. (1951) The pre-erythrocytic stage of *Plasmodium falciparium* Trans Roy Soc Trop Med & Hyg 44: 405-419.

Shouval, D., Ligumsky, M. and Ben-Ishay, D. (1978). Effect of co-trimoxazole on normal creatinine clearance. Lancet 2:244-245.

Singleton, J. W., (1977). National Cooperative Crohn's Diseases Study (NCCDS). Results of drug treatment. Gastroentology. 72, A110/1133.

Sirima, S. B., Konate, A., Tiono, A. B., Convelbo, N., Cousens, S. and Pagnoni, F. (2003). Early treatment of childhood fevers with pre- packaged anti malarial drugs in the home reduces severe malaria morbidity in Burkina Faso. Tropical Medicine and International Health: 8, 133 -139.

Skinner-Adams, T. S., Andrews, K. T., Melville, L., McCarthy, J. and Gardiner, D. L. (2007). "Synergistic interactions of the antiretroviral protease inhibitors saquinavir and ritonavir with chloroquine and mefloquine against *Plasmodium falciparum in vitro*," Antimicrobial Agents and Chemotherapy, 51(2): 759–762.

Skinner-Adams, T. S., McCarthy, J. S., Gardiner, D. L., Hilton, P. M. and Andrews, K. T. (2004). "Antiretrovirals as antimalarial agents," Journal of Infectious Diseases 190(11):1998–2000.

Smith, D .K., Grohskopf, L. A., Black, R. J., Auerbach, J. D., Veronese, F., Sruble, K. A., Cheever, L., Johnson, M., Paxton, L. A., Onorato, I. M. and Greenberg, A.E. (2005) Antiretroviral Post-exposure prophylaxis After Sexual Injection – Drug Use, or other Non occupational Exposure to HIV in the United States. DHHS Recommendations and Reports January 21, 2005/54 (RR02) Atlanta, Georgia.

Smith, P. F., Ogundele, A., Forrest, A., Wilton, J., Salzwedel, k., Doto, J., Allaway, G. P., and Martin, D. E. (2007). Phase I and II study of the safety, virologic effect, and pharmacokinetics/pharmacodynamics of single dose 3 – 0 (3/ 3/ - dimethylsuccinyl) betulinic acid (bevirmat) against human immunodeficiency virus infection. .Antimicrob Agents Chemother. 51 :3574 - 3581

Stevens, L. (2001) Sexual assault post exposure: putting policy into action. New York State Department of Health, New York AIDS Institute HIV Medical Alert New York, 5: 1-7.

Stickney, R., Simmons, W. S., De Angelis, R. L., Rundles, R .W. and Nichol, C. A. (1973). Pharmacokinetics pf pyrimethamine (PRM) and 2, 4-diamino-5-(3^1, 4^1-dichlophenyl)-6-methyl pyrimidine (DMP) relevant to meningeal leukemia. Proc. Am. Assoc. Cancer Res.14: 52.

Summers, R. W., Switz, D. M., Sessions, J. T. Jnr., Beckel, J. M., Best, W. R. Kern, F. Jr., and Singleton, J. W. (1979). National Cooperative Crohn's Disease Study: results of drug treatment. Gastroenterology. 77: 847 – 869.

Taha, T. E., Kumwenda, N. I., and Gibbons, A. (2003) Short post exposure prophylaxis in newborn babies to reduce mother-to-child transmission of HIV-1-: NVAZ randomized clinical trial. Lancet, 362 (9391): 1171-1177.

Takken, W., (2002) Do insecticide-treated bed nets have an effect on malaria vectors? Tropical Medicine and International Health 7:1022 – 1030.

ter Kuile, F. O., Parise, M. E.,Verhoeff, F. H., Udhayakumar, V., Newman, R. D., van Eijk, A. M., Rogerson, S. J. and Steketee, R. W.(2004). The burden of co-infection with human immunodeficiency virus type 1 and malaria in pregnant women in sub-Saharan Africa Am J Trop Med Hyg 71:41 – 54.

Thompson, G. R. (1963) Malaria and stress in relation to haemoglobin S and C Br. Med J 2: 976-978.

Tiskoff, S. A., Varkonyi, R., Cahinhinan, N. Abbes, S., Argyropoulous, G., Destro –Bisol, G., Drousiotou, A., Dangerfield, B., Lefranc ,G., Loiselet, J., Piro, A., Stoneking, M., Tagarelli,A., Tagarelli, G., Touma, E. H., William, S. M., and Clark, A. G .(2001) Halo type diversity and linkage disequilibrium at human G6PD: recent origin of alleles that confer malarial resistance. Science 293:455 – 462.

Tracy, J. W. and Webster, L. T. (2001) Drugs used in the chemotherapy of protozoal infections**:** Malaria. In: Goodman and Gilman's The Pharmacological basis of Therapeutics. Hardman J.G., Limbirds L., Gilman A. G. (eds) McGram Hill Publishing Division, New York, 10th edition,pp. 1069 -1095.

Tsoka, J. M., Sharp, B.L. and Kleinschmidt, I. (2002) Malaria mortality in a high-risk area of South Africa. Presented at Third MIM Pan-American malaria conference: Global advances in malaria research: Evidence-based decision making for malaria control policy. Abstract 528. Arusha, Tanzania, 2002 November.

United Kingdom Group of Transmitted HIV Drug Resistance (2005). Time Trends in primary resistance to HIV drugs in the United Kingdom: multicentre observational study. B. M. J. 331: 1368-1371.

United Nations Children's Education Fund. (2003), UNICEF malaria technical notes, no 6, February 2003

United Nations Development Programme (2008). Human and Income poverty: developing countries. In: UNDP 2007/2008 Human Development Reports.

United Nations High Commission for Refuges (2005). Community Conversations in Response to HIV/AIDS: A capacity building project with refugees and the host population. Republic of Congo. UNHCR HIV/AIS No 3, Geneva, Switzerland.

United Nations Programme on HIV/AIDS (2004). AIDS epidemic update. UNAIDS, Geneva,

United Nations Programme on HIV/AIDS (2007). "AIDS epidemic update." UNAIDS and WHO, Geneva.

United Nations Programme on HIV/AIDS (2008). Report on the global AIDS epidemic.UNAIDS, Geneva.

United Nations Programme on HIV/AIDS (2009). AIDS epidemic update.UNAIDS and WHO, Geneva.

United States President's Emergency Plan for AIDS Relief (2008). The Power of Partnerships: Annual reports to congress.

Naing , U. T., Win, U. H., Nwe, D. Y., Myint, U. P. T. and Shwe U.T. (1988). The combined use of artemether, sulfadoxine and pyrimethamine in the treatment of uncomplicated falciparium malaria. Transactions of the Royal Society of Tropical Medicine and Hygiene 82:530 -531.

Van Eijk, A. M., Ayisi, J. G., ter Kule, F .O., Otieno, J. A., Misore, A. O., ooni J. O., Rosoen, D. H., Kager, P. A., Steketee, R. W. and Nahlen, B. L. (2004).

Effectiveness in intermittent preventive treatment with sulphadoxine e-pyrimethamine for control of malaria in pregnancy in western Kenya: hospital-base study. Trop Med Int Health 9(3): 351-360.

Van Geertruyden, J-P., Thomas, F., Erhart, A. and D'Alessandro, U. (2004). The contribution of malaria in pregnancy to perinatal mortality Am J Trop Med Hyg 71 (2 suppl): 35 - 40

Vlahov, D., Graham, N., Hoover, D., Flynn, C., Bartlett, J. G. and Margolick, J. B. (1998). Prognostic markers for AIDS and infectious disease death in HIV-1 infected injection drug users: plasma viral load and CD_4+ cell count. JAMA; 279:35-40

Wenzel, R. P., Hunting K, J., Ostermary, C. O. and Sande, M. A. (1976). Providentia Stuartii, a hospital pathogen: potential factors for its emergence and transmission. Am J. Epidemiol., 104: 170-180.

White, M. G. and Asch, M. J. (1971). Acid-base effects of topical mafenide acetate in the burned patient. N. Engl. J. Med. 284: 1281 – 1286.

White, N. J. (1994). Clinical pharmacokinetics and pharmacodynamics of artemisinin and derivatives. Trans R Soc Trop Med Hyg 88: 41-43.

White, N. J., Nosten, F., Looareesuwan, S., Watkins, W. M., Marsh, K., Snow, R. W., Kokwaro, G., Ouma, J., Hien, T. T., Molyneux, M. E., Taylor, T. E., Newbold, C. I., RuebushII, T. K., Danis, M., Greenwood, B. M., Anderson, R. M., and Olliar, P. (1999). Averting a Malaria disaster. Lancet 353: 1965-1967.

Whitworth, J. (2006). Malaria and HIV. HIV Insite Knowledge Base Chapter. San Francisco, University of California Center for HIV Information.

Whitworth, J., Morgan, D., Quigley, M., Smith, A., Mayanja, B., Eotu, H., Omoding, N., Okongo, M., Malamba, S. and Ojwiya, A. (2000). Effect of HIV-1 and increasing immuno suppression on malaria parasitaemia and clinical episodes in adults in rural Uganda: a cohort study. Lancet 356:1051-1056

Wiktor, S. Z., Ekpini, E. and Karon J. M. (1999). Short-course oral zidovudine for prevention of mother-child transmission of HIV-1 in Abidjan, Côte d'Ivoire: a randomized trial Lancet 353: 781-785

Wilkinson, G. R.(2001). Pharmacokinetics: The Dynamics of Drug Absorption, Distribution, and Elimination In: Goodman and Gilman's The Pharmacological basis of Therapeutics. Hardman J.G., Limbirds L., Gilman A. G. (eds) McGram Hill Publishing Division, New York, 10th edition, pp. 3-29.

Willcox, M. L. and Bodeker, G. (2004). Traditional herbal medicines for malaria. BMJ 2004; 329: 1156-9.

Winkler, C., Wirleitner, B., Schroecksnadel, K., Schennach, H., Mur, E. and Fuchs, D. (2004). *In vitro* effects of two extracts and two pure alkaloid preparations of *Uncaria tomentosa* on peripheral blood mononuclear cells. Planta Med 70: 205-10

Winkler, C., Neurauter, G., Schroecksnadel, K., Wirleitner, B. and Fuchs, D. (2005). Immunomodulatory effects of plant extracts in Drug Development from New

Molecules - Recent Progress in Medicinal Plants, Govil J.N., (ed) vol. 11, Studium Press LLC, Houston, Texas,

World Health Organization (1981). Chemotherapy of malaria. WHO Monograph Series No. 27, 2nd ed., WHO, Geneva

World Health Organization (2004a). Malaria and HIV/AIDS interactions and implications: conclusions of a technical consultation convened by WHO; 2004, 23–25 June. Report no.: WHO/HIV/.08. Geneva;

World Health Organization (2004b). A Strategic Framework for Malaria Prevention and Control during Pregnancy in the Africa Region. Brazzaville: WHO Regional office for Africa, AFR/MAL/04/01.

World Health Organization (2004c). The role of laboratory diagnosis to support malaria disease management. Focus on the rapid diagnostic tests in areas of high transmission. Report of a WHO Technical Consultation, pp 1-40.

World Health Organization (2004d). Joint WHO/UNAIDS/UNICEF statement on use of co-trimoxazole as prophylaxis in HIV exposed and HIV infected children. Nov 22

World Health Organization (2005). Malaria control today: Current WHO recommendations. Working document. Roll Back Malaria Department. WHO, Geneva.

World Health Organization (2006). Guidelines for the treatment of malaria, volume 2. Global Malaria Programme Geneva, Switzerland

World Health Organization (2007). WHO Global Malaria Programme. Global AMDP database-AFRO. Geneva, Switzerland.

World Health Organization (2008). Global Malaria Control and Elimination: report of a technical review 17^{th}--18^{th} January, WHO, Geneva, NLM classification: WC765.

World Health Organization (2009). Malaria Microscopy Quality Assurance Manual Vrsion 1.Malaria light microscopy: Creating a culture of Quality. WHO,Geneva.

Xiao L., Owen S. M., Rudolph, D. L., Lal, R.B., and Cal, A. A., (1998). Plasmodium falciparium antigen-induced human immunodeficiency virus type 1 replication is mediated through induction of tumour necrosis factor-alpha. J. Infect Dis. 177: 437-445.

Yadav, A., Fitzgerald, P., Sajadi, M. M., Gilliam, B., Lafferty, M. K., Redfield, R. and Reid, W. (2009). "Increased expression of suppressor of cytokine signaling-1 (SOCS-1): a mechanism for dysregulated T helper-1 responses in HIV-1 disease," Virology 385(1): 126–133

Yousif, M. A. and Adel, A. A. (2000). Antimalarials prescribing patterns in Gerzira State: precepts and practices. Eastern Mediterranean Health Journal 6: 939-947