COMPARISON OF MALARIA PARASITAEMIA AMONG CHILDREN LESS THAN FIVE YEARS LIVING WITH HIV AND THOSE WITHOUT HIV IN HEALTH FACILITIES IN JOS, NIGERIA

BY

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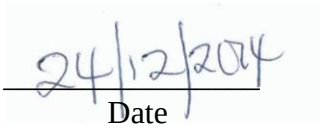
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ATTESTATION

I declare that the work in the thesis entitled "Comparison of Malaria Parasitaemia among Children less than Five Years Living with HIV and those without HIV in Health Facilities in Jos" was performed by me in the Department of Community Medicine, Ahmadu Bello University, Zaria under the supervision of Dr Saad Ahmed. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at any university.

Olawuyi Kayode Abraham  Signature  Date

CERTIFICATION

I certify that the work for this thesis entitled "Comparison of Malaria Parasitaemia among Children less than Five Years Living with HIV and those without HIV in Health Facilities in Jos" by Olawuyi Kayode Abraham meets the regulations governing the award of the degree of Masters of Public health in Field Epidemiology of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to the Almighty God who gave me life and to my lovely wife- Foluke Olawuyi and son- OgoOlorun Olawuyi for their support during the course of this work.

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LIST OF ACRONYMS

ACT	-	Artemisinin Based Combination Therapy
ART	-	Antiretroviral Therapy
HIV	-	Human Immunodeficiency Virus
IPTp	-	Intermittent Preventive Therapy in Pregnancy
ITN	-	Insecticide Treated Bed Net
MP	-	Malarial Parasitaemia
MPD	-	Mean Parasite Density
RBM	-	Roll Back Malarial
RDT	-	Rapid Diagnostic Test
UNICEF	-	United Nations Children Education fund
WHO	-	World Health Organization

DEFINITION OF TERMS/OPERATIONAL DEFINITION

Malaria parasitaemia: This refers to the presence of at least one asexual parasite in the blood film of the study participant

Parasite density: Refers to the number of parasites per microlitre of blood, taking the putative mean number of leucocytes per microlitre of blood as 8000 expressed as:

$$\text{Parasite density} = \frac{\text{Parasite count} \times 8000}{\text{Number of WBC counted}}$$

Caregiver's Knowledge: Knowledge is scored on a five point domain, one point for correct response and zero for wrong response. Thus maximum score is five and minimum is zero. Anyone with 2 points and below is graded as having poor knowledge, while scores of 3 points and above is graded as good knowledge.

Fever: Fever is defined as axillary body temperature $\geq 37.5^{o}C$ and measured using digital clinical thermometer.

Anaemia: Anaemia is defined as haemoglobin < 11g/dl. Haemoglobin normal range for under five children is 11-13g/dl [152]

Stable malaria transmission: Areas with a stable transmission have a persistent transmission and hence prevalence of infection

Unstable malaria transmission: In areas with an unstable malaria transmission the prevalence of infection varies highly over time and space

SUMMARY

Malaria and HIV are among the two most important diseases that pose a great challenge to human health. In Nigeria malaria contributes significantly to the burden of disease especially among children less than five years. Despite all efforts aimed at prevention and control of malaria, its prevalence is still relatively high in Jos. This study was therefore conducted to determine the prevalence and circulating species of malaria parasites in under fives as well as assessing the factors associated with malaria parasitaemia among HIV positive and HIV negative under five children in selected health facilities in Jos.

A comparative cross sectional study was conducted among children less than five years living with HIV and those without HIV. Blood specimen was collected and tested for malaria parasitaemia by microscopy and RDT. Haemoglobin estimation was carried out and CD4% determined for children living with HIV. Structured questionnaire was used to obtain demographic information and potential factors associated with malaria parasitaemia.

A total of 528 children less than 5 years old were enrolled, 264 were living with HIV while the other half were HIV negative. The mean age (± standard deviation) of children living with HIV was 40.6 (± 13.7) months, while the mean age (± standard deviation) of children less than 5 years who are HIV negative was 28.7 (± 14.2) months. The prevalence of malaria parasitaemia among HIV positive children was 16%, while among HIV negative children was 23.1%.The most predominant species found among under five children who participated in this study was *Plasmodium falciparum*. A co infection of *Plasmodium falciparum* and *Plasmodium malariae* was also observed among children living with HIV. Important environmental factors significantly associated with parasitaemia in this study was the presence of grasses around the house where the child reside (OR, 3.0; 95% CI, 1.4 -11) and pools of stagnant water close to the house (OR,

9.0; 95% CI, 1.3-85). The presence of mild anaemia was significantly associated with malaria parasitaemia among HIV negative children (OR, 2; 95% CI, 1.7-3.3). Ninety five percent of caregivers of children living with HIV had good knowledge of fever, malaria infection and management, while 97.7% of caregivers of HIV negative children also had a good knowledge.

The prevalence of malaria parasitaemia was low among the study population. This is probably due to the seasonal variation in malaria parasitaemia intensity and the good knowledge of caregivers on malaria infection and management. Hence there is need to sustain the current media and health facility based campaigns on malaria prevention strategy and maintenance of clean environmental condition.

Key Words: Prevalence, Malaria parasitaemia, Children, HIV positive

CHAPTER ONE - INTRODUCTION

1.1 Background Information

Malaria is a mosquito-borne infectious disease. It is the 3rd leading cause of death for children under five years of age (U5) worldwide, after pneumonia and diarrheal disease.[1] Globally, the World Health Organization (WHO) estimates that in 2010, 216 million clinical cases of malaria occurred, and 655,000 people died of malaria, 86% of them were children in Africa.[2] Two to four episodes of malaria have being reported to occur annually among children less than 5 years.[3] Nigeria, Democratic Republic of Congo (DRC), Ethiopia, and Uganda accounts for nearly 50% of the global malaria deaths.[4] Almost 1 out of 5 deaths of children U5 in Africa is due to malaria.[5] In Nigeria malaria accounts for 60 percent of outpatient visits to health facilities, and 30 percent hospitalization. The National Malaria Control Program reported that 30 percent U5 mortality is attributable to malaria infection in Nigeria.[6] Malaria has the greatest prevalence, close to 50%, in children aged 6-59 months in the South West, North Central and North West regions of the country.[7] It is estimated that about 40 million people are living with HIV worldwide, of these 2.3 million are children under the age of five.[8] In Sub-Saharan Africa it is estimated that about 25 million children and adults are living with HIV while Nigeria has the second largest number of people living with HIV (PLWH) (3.1 million) after South Africa (5.6 million).[9,10] Few studies have shown an interaction between HIV and malaria infection especially in children, while others were inconclusive about the interaction.[11-13] The two diseases together contribute immensely to the burden of under five mortality in Nigeria.[14] Studies have shown that malaria is a stimulator of the immune system and individuals expose frequently to malaria have

enhanced serum levels of immnunoglobulins and an accelerated rate of IgG turnover.[12] Therefore, malaria might have an adverse effect on HIV infection both by stimulating the T-cell turnover and by impairing the T-cell cytotoxic function.[15] *Plasmodium* parasitaemia differs in instances of asymptomatic and clinical malaria, and the degree of parasitaemia may influence the pathological and biochemical presentations of individuals presenting with either of these conditions.[16,17] Reports have shown that in most cases of clinical malaria, anaemia is a prominent factor which is caused by destruction of infected blood cells by the reticuloendothelial system and haemolysis of infected cells.[18,19]

Malaria infection is caused by plasmodium parasites namely: *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae, and recently Plasmodium knowles.*[20] In a systematic review of publications on *plasmodium* infections in Africa,[21] it was observed that malaria related fever among under five children is on the decline, consequently the World Health Organization recommends that malaria treatment should begin with parasitological diagnosis.[22-26] This is cost effective and offers practical means of improving malaria diagnosis and treatment especially in areas of low transmission.[27,28] Although there is still no consensus on laboratory-confirmed diagnosis versus presumptive treatment in endemic areas.[25,29] It is important for clinicians to base their treatment decisions on laboratory confirmed cases of malaria because parasitological diagnosis will enable clinicians to report more accurately whether a patient presenting with fever or history of fever is actually parasitaemic.[25] Reliance on laboratory result will also reduce the unnecessary use of antimalarials in non-malaria cases, and improving treatment for other potentially fatal causes of fevers.

1.2 Problem Statement

The World Health Organization estimated that 3-5 million U5 children die from malaria related causes every year globally.[1] Greater than 1 million children die annually from malaria infection from Africa, 70% of these deaths occur in children less than five years old [30] In Nigeria it is estimated that greater than 300,000 children age less than five years die from malaria related causes annually.[7] National Malaria Control Program reported that 30% U5 mortality is due to malaria infection in Nigeria.[6] Hence Nigeria's under five mortality ranked 9th in the world. Direct indicators to this is the fact that only 6% of Nigeria under 5 children sleep under insecticide treated nets and only 33% receive antimalarial drugs.[7]

An estimated 33 million people including children less than five years are currently living with HIV worldwide, with approximately 22.5 million cases occurring in sub-Sahara Africa where it the leading cause of death.[31] In Nigeria greater than four million persons are living with HIV/AIDS with North Central having the highest levels of infection in the Country.[32] Malaria contributes to a temporary increase in viral load among HIV infected individuals, this worsen the clinical disease especially in children less than five years.[33] According to the Nigeria malaria indicator survey, North central had the 2nd greatest prevalence of malaria among children 6-59 months.[7]

Malaria control and interventions have been implemented and in the recent past intensified as an effort to attain the World Health Assembly, Roll Back Malaria, and Millennium Development Universal targets with the aim of reducing and interrupting disease transmission in sub Saharan Africa. Jos is a malaria endemic area in which malaria control measures such as the use of Artemisinin Combined Therapy (ACT), the

use of insecticide treated bed nets (ITNs), and Intermittent Preventive Treatment (IPTp) for pregnant women and children have been implemented. Despite of all these efforts yet the overall prevalence of malaria infection from studies recently conducted in Jos remains high among the under-five children: (38-56%).[34-36] Thus it is very important that research be conducted to identify the gaps. Therefore this work is necessary to confirm the prevalence and circulating species of malaria parasitaemia in under-fives, as well as assessing the factors (socio-demographic, environmental,) associated with malaria parasitaemia in Jos, North Central Nigeria

1.3 Justification

Despite the availability of the malaria control measures and intervention, the morbidity and mortality in under-fives is still unacceptably high. Also there is paucity of data on the interaction of malaria and HIV among children less than five years.

The World Health Organization recommends the parasitological diagnosis and confirmation of malaria before treatment.[26] However this is usually not adhered to by health care providers. Therefore the data generated will provide information on the parasitological diagnosis and factors associated with prevalence of malaria parasitaemia in Jos.

1.4 Research Questions

1. What is the prevalence of malaria parasitaemia among children under five years living with HIV and those without HIV?
2. What are the factors associated with malaria parasitaemia among children under five years living with HIV and those without HIV in Jos?

3. What is the level of knowledge of caregivers of children under five years on fever, malaria infection and management?

1.5 General Objective and Specific Objectives

1.5.1 General objectives

To determine the prevalence and assess factors associated with malaria parasitaemia among children less than five years living with HIV and those without HIV in selected health facilities.

1.5.2 Specific objectives

1. To determine the prevalence of malaria parasitaemia among children less than 5 years living with HIV and those without HIV in selected health facilities.
2. To identify species of malaria parasites among children less than 5years living with HIV and those without HIV in selected health facilities.
3. To determine factors associated with malaria parasitaemia among children less than 5years living with HIV and those without HIV in selected health facilities.
4. To assess caregivers' knowledge of fever, malaria infection and management among children less than 5 years in the selected health facilities.
5. To determine the relationship between haemoglobin estimation, CD4% and malaria parasitaemia among children less than 5years in the selected health facilities

CHAPTER TWO - LITERATURE REVIEW

2.1 Historical Perspective

The term malaria originates from Medieval Italian : *mala aria* — “bad air”; the disease was formerly called *ague* or *marsh fever* due to its association with swamps and marshland.[37] Malaria was once common in most of Europe and North America,[38] where it is no longer endemic,[39] though imported cases do occur.[40] Scientific studies on malaria made their first significant advance in 1880, when Charles Louis Alphonse Laveran—a French army doctor working in the military hospital of Constantine in Algeria—observed parasites inside the red blood cells of infected people for the first time. He therefore proposed that malaria is caused by this organism, the first time a protist was identified as causing disease.[41] A year later, Carlos Finlay, a Cuban doctor treating people with yellow fever in Havana, provided strong evidence that mosquitoes were transmitting disease to and from humans.[42] This work followed earlier suggestions by Josiah C. Nott,[43] and work by Sir Patrick Manson, the "father of tropical medicine", on the transmission of filariasis.[44]

A Scottish physician Sir Ronald Ross in 1898 proved that mosquito was the vector for malaria in humans by showing that certain mosquito species transmit malaria to birds. He isolated malaria parasites from the salivary glands of mosquitoes that had fed on infected birds.[45] The first effective treatment for malaria came from the bark of cinchona tree, which contains quinine. This tree grows on the slopes of the Andes, mainly in Peru. Theindigenous peoples of Peru made a tinture of cinchona to control fever. Its effectiveness against malaria was found and the Jesuits introduced the treatment to Europe around 1640; by 1677, it was included in the London as an antimalarial

treatment.[46] It was not until 1820 that the active ingredient, quinine, was extracted from the bark, isolated and named by the French chemists Pierre Joseph Pelletier and Joseph Bienaime Caventou.[47,48] Quinine become the predominant malarial medication until the 1920s, when other medications began to be developed. In the 1940s, chloroquine replaced quinine as the treatment of both uncomplicated and severe malaria until resistance supervened, first in Southeast Asia and South America in the 1950s and then globally in the 1980s.[49] Artemisinins, discovered by Chinese scientist Tu Youyou and colleagues in the 1970s from the plant Artemisia annua, became the recommended treatment for *P. falciparum* malaria, administered in combination with other antimalarials as well as in severe disease.[50]

2.2 Conceptual Framework

The conceptual frame work represents a relationship between factors associated with malaria transmission and prevalence among children less than five years. Several factors influence the transmission and prevalence of malaria parasitaemia among children less than five years. These factors can be grouped into environmental, biological, individual factors, control activities, land use activities and HIV infection.

The distribution, seasonality and transmission intensity of malaria is affected by environmental factors.[156] Important environmental factor associated with malaria transmission is the conditions for breeding and survival of the anopheles mosquitoes. These includes water, minimum temperature and humidity that allow them to survive long enough for the vector stage of the parasite's life cycle to be completed in about 10 days. These factors are influenced by climates, topography, soil conditions, vegetation cover, land use activities and water. Consequently climate changes, land use activities

e.g. water management, agriculture, urbanization and deforestation can lead to significant increase or decrease in malaria transmission.[51]

Individual factors which include socio demographic, access to health facilities, knowledge on malaria infection and management contributes significantly to the prevalence of malaria. In a study conducted by Mclntyre *et al*[52], poverty and ignorance of people contributes in creating source and spread of malaria and hinder disease control strategy. Also Onwujekwe *et al*[53] in 2010 reported that poorest groups in a society did not seek care as much as the rich. Economic inequalities in areas such as the control of household resources also affects access to Insecticide treated nets and usage. In another study in Nigeria,[54] women explained that since they were financially dependent on their husbands, they were not able to purchase ITNs for themselves and their children.

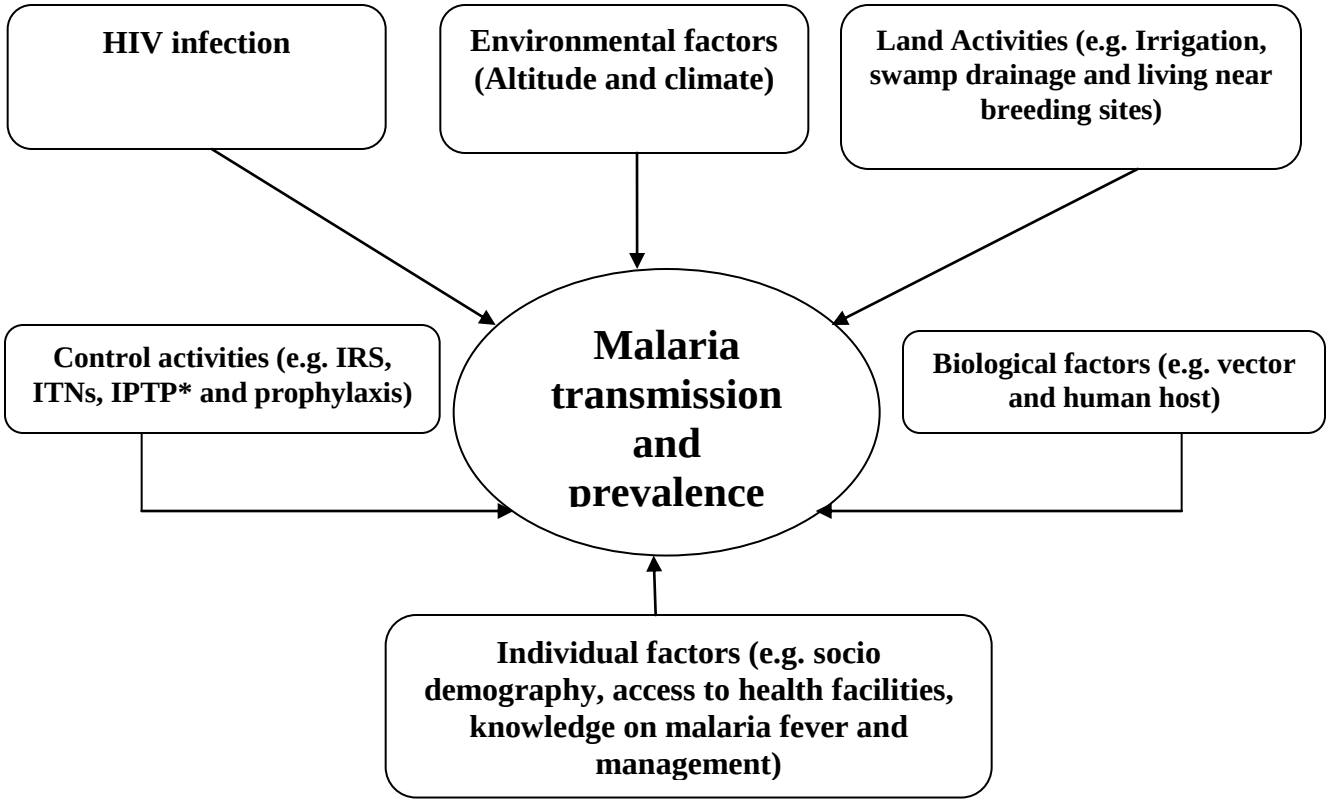
Alaba,[55] also observed that malaria afflicts primarily the poor who tend to live in dwellings that offer little or no protection against mosquitoes. Furthermore Teklehaimanot *et al*[56]observed from their study that "the continuation of brutal poverty and hunger in much of the world is undoubtedly linked to large numbers of unnecessary deaths from malaria especially among children less than five years".

Malaria control activities include both vector control and use of prophylaxis. The roll back malaria[57] in 2010 reported that ITN use reduces human contact with infected mosquitoes, and have been shown to be an effective malaria prevention measures. Children less than five years who sleeps under an insecticide treated nets were less likely to have malaria parasitaemia in their peripheral blood. ITN use could prevent approximately 7% of the global under five mortality.[2]

The UNICEF[58] corroborates that under five mortality rates could be reduced by almost 25-30% if all young children in malaria endemic areas were protected by treated bednets at night.

Malaria contributes to a temporary increase in viral load among HIV infected individuals, this worsen the clinical disease especially in children less than five years.[33]

Despite all these control efforts, Abeje *et al.*[59] reported that only 3% of African children sleep under treated nets while only about 20% sleep under any other kind of nets. This accounts for high rate of mortality amongst children due to malaria and its related challenges.



*IPTP=intermittent preventive therapy in pregnancy, IRS= indoor residual spraying, ITNs= insecticide treated nets

Figure 1: Conceptual framework for transmission and prevalence of malaria parasite

2.3 Epidemiology of Malaria and Human Immunodeficiency Virus (HIV)

Malaria and HIV are among the two most important diseases that pose a great challenge to human health.[60] The World Health Organization reported an estimated 40 million people living with HIV worldwide and there are 350-500 million clinical malaria episodes yearly.[31] These two diseases together cause more than 4 million deaths in a year globally. Seventy percent (70%) of individuals in Sub Saharan Africa are reported to be infected with HIV, while 80% of malaria cases in the world occur in the same region.[61-62] Inspite of all efforts and resources directed towards the eradication of malaria worldwide, this ancient disease still constitute a huge public health problem especially in Sub-Saharan Africa.[63] Malaria is reported to have occurred in about 109 countries with about 40% of the world's population at risk. It is most common between 23.5^0 North latitude and 23.5^0 South of latitude, which include regions of Africa, Asia, South America, the Caribbean and some isolated parts of North America.[64]

The World Health Organization reported that there were an estimated 207 million cases of malaria in 2012 (uncertainty range: 135 – 287 million) and an estimated 627 000 deaths (uncertainty range: 473 000 – 789 000), while 90% of all malaria deaths occur in sub-Saharan Africa.[63] In 2012, malaria killed an estimated 482 000 children under five years of age. That is 1300 children every day or one child almost every minute.[63] Though malaria is not endemic in Europe and America, in a review Taylor,[65] reported 10,000 cases of malaria occurred in Western Europe and 1300-1500 in United States. Majority of these cases were imported from malaria endemic zones into these countries.[66] In Africa an estimated 16% of all deaths among under five children was due to malaria.[67] Maternal malaria is associated with up to 200,000 estimated infant deaths yearly in Sub-Saharan

Africa.[68] In Nigeria malaria contributes significantly to the burden of disease especially in communities where it is endemic and in quantifying the economic burden of malaria in the country, Jimoh *et al* reported that malaria is responsible for annual economic loss of 132 billion naira in treatment and loss in man-hour.[69] Its accounts for nearly 110 million clinically diagnosed cases per year, 60 percent of outpatient visits, and 30 percent hospitalization.[70] In addition, at least 50% of the population has at least one episode of malaria annually.[7] This result in high productivity losses while children that are less than 5 years have 2-4 attacks annually.[71] Pregnant women and children are particularly vulnerable to malaria attacks because of their low levels of immunity, while about 125 million pregnant women are at risk of infection each year.[72] The Federal Ministry of Health and the National Malaria Control Program reported that 11 percent maternal mortality, 25 percent infant mortality and 30 percent under five mortality is attributable to malaria infection in Nigeria. [69,70]

In Sub-Saharan Africa an estimated 25 million children and adults are living with HIV.[9] In Nigeria about 4.3 million individuals are reported to be living with HIV, with the North Central region having the highest HIV infection in the country.[32] Earlier studies failed to show an association between *P. falciparum* and HIV infection in children probably due to inherent limitation in the study design.[73,74] Some other studies were inconclusive on the interaction of malaria and HIV among children living in areas of stable malaria epidemiology.[75] Recent studies have shown that an association exists between HIV infection and *P. falciparum* malaria among children less than five years of age.[11-13] An association between HIV-associated immune-suppression and the severity of clinical malaria in children was observed in a prospective cohort study in Uganda.[14] Also

advanced immunosuppression in HIV –infected children resulted in more episodes of clinical malaria and higher parasite densities compared with HIV-infected children whose immune status was less compromised.[76] In another report Mermin[74] showed that malaria was more common among HIV-infected children less than 5 years old than among HIV-uninfected children. A higher mortality was found among HIV –infected children with malaria treated with chloroqiune compared to HIV-uninfected children.[77] In systematic review, it was found that the levels of parasitaemia, parasite densities; the risk of anaemia and hospitalization due to malaria were higher among children less than five years old living with HIV.[78] A study conducted in Uganda showed that children living with HIV with severe malarial anaemia suffered all-cause and malaria-related mortality than children not infected with HIV.[79] Other studies also suggest that HIV is associated with hospital admission for severe malaria, clinical cerebral malaria and higher parasite densities in children.[76,80] However, no association has been found between clinical malaria and low CD4 T-cell counts, [81] in accordance with previous studies showing that lower CD4 T-cell counts were associated with higher incidence of pneumonia, sepsis and TB, but not malaria.[82] Although these associations were found in children but not infants, suggesting that HIV might stunt the age-related acquisition of natural immunity to malaria, thus having little effect among the youngest children who have not yet acquired this immunity.[82]

2.4 Prevalence of Malaria Parasitaemia among Children less than Five Years

The prevalence and burden of malaria differ from one region to another depending on the endemicity of the disease.[60] In a study conducted in Nyasa Health Center in Tabora region of Western Tanzania among 300 under five children with fever or history of fever

in the past 2 weeks, Mazigo *et al*[83] reported a prevalence of 12% with *Plasmodium falciparum* been the predominant species found. Although in a study from Gabon a decline in prevalence of *Plasmodium falciparum* among children below five years from 31.2% in 2005 to 18.3% in 2008 was observed.[84] Other studies reported similar low prevalence of malaria and HIV co infection. [80,85] A prevalence of 54.6% was reported from a Health facility based study in Uganda which was higher than previous report.[86] In a community based survey of malaria parasitaemia among asymptomatic apparently healthy under five children in Jos, Nigeria a prevalence of 38%[34] was reported, while earlier studies conducted in Jos in 2008, reported a prevalence of 56.9%[35] In a recent study conducted among under five children in health facilities in Jos, a malaria parasitaemia prevalence of 48.06%[36] was found. Studies conducted in Eastern Nigeria on prevalence of malaria parasitaemia gave a high prevalence of 76.4% and 80.3% respectively. These studies were conducted during the rainy season which is a high malaria transmission season and in rural community which lies within the humid tropical rain forest with Guinea Savanna Vegetations. [87,88] The prevalence of malaria parasitaemia among individuals living with HIV including children from Osogbo, South Western Nigeria was 18.5%.[89] In another study conducted in Ota, Ogun State, South West Nigeria, 47.9% prevalence of *Plasmodium falciparum* was reported among under five HIV negative children.[90] This finding was similar to the report from the Nigeria malaria indicator survey that reported 42% prevalence of malaria parasitaemia among under five in 2010.[7] He further reported that children 2-3 years old had the highest percentage (67.5%) of *Plasmodium falciparum* infections.[90]In a study in Rwanda, *Plasmodium falciparum* was seen in one out of six children under five years of age. A prevalence of

11.7% and 16.7% was identified by microscopy and PCR respectively. Jean-Bosco *et al* further reported that most of the infections were asymptomatic with anaemia observed in 82% of the children with parasitaemia.[91]

2.5 The Causative Agent of Malaria

Malaria is a mosquito-borne infectious disease of human and other animals caused by *protists* (a type of microorganism) of the genus *Plasmodium* (*pylum Apicoplexa*). [92] Malaria in human is caused by five species of *plasmodium* parasites namely *Plasmodium vivax, Plasmodium malariae, Plasmodium falciparum, Plasmodium ovale and recently Plasmodium knowlesi*, a zoonotic specie prevalent in South Asia. [92] The tropical zone is the endemic home of all malaria parasites. *Plasmodium falciparum* is the most common species in the tropical and subtropical areas of Africa, and parts of Central America and South America, Bangladesh Pakistan, Afghanistan, Nepal, Sri Lanka, Southeast Asia, Indonesia, and Philippines. This specie is the most dangerous, accounting for half of all clinical cases of malaria and 90 percent of deaths from the disease. It is transmitted primary during raining season.[93]

Plasmodium vivax is the most widely distributed parasite existing in temperate as well as subtropical area. It is found in South America, Africa, India and Pakistan. *Plasmodium malariae* has much lower prevalence than *P. falciparum and P. vivax*. It is found in tropical and subtropical regions. In tropical Africa it accounts for up to 25% *plasmodium* infections. *P. ovale* is relatively rare parasite, restricted to tropical climate in West and East Africa. [94,95]

2.6 Transmission and Life Cycle of Malaria Parasite

The *plasmodium* parasites undergo many stages of development and their life cycle is completed in two hosts, viz; humans (intermediate host) and mosquitoes (definitive host). Malaria parasites are transmitted to humans by the bites of an infected female Anopheles mosquito. Sporozoites contained in the saliva of the mosquito are inoculated into the blood of human host when the mosquito takes a blood meal. Infection can also occur by transfusion of infected donor blood. Occasionally congenital transmission occurs when a mother is non-immune.[96] These Sporozoites travel through the blood to the liver where they invade red blood cells (RBC). Inside the RBC the merozoites multiply rapidly until they force the red cell to burst, releasing into the blood stream new generation of merozoites that go to infect other RBCs. At this time the clinical symptoms of overt malaria in the infected individual become apparent. Some merozoites divide to form gametocytes, i.e. immature male and female gametes. If another mosquito bites the human and ingests these gametocytes, the life cycle of the malaria parasite begins again.[97] At this point the male and female gametocytes fuse and form a ookinete- a fertilized, motile zygote. Ookinete develop into new Sporozoites that migrate to the insect's salivary glands, ready to infect a new vertebrate host. The Sporozoites are injected into the skin, in the saliva, when the mosquito takes a subsequent blood meals.[98] Only female mosquito feed on blood; male mosquitoes feed on plant nectar, and thus do not transmit the disease. The female of the *Anopheles* genus of mosquito prefer to feed at night. They usually start searching for meal at dusk, and will continue throughout the night until taking a meal. Malaria parasite can also be transmitted by blood transfusion, although this is rare.[96]

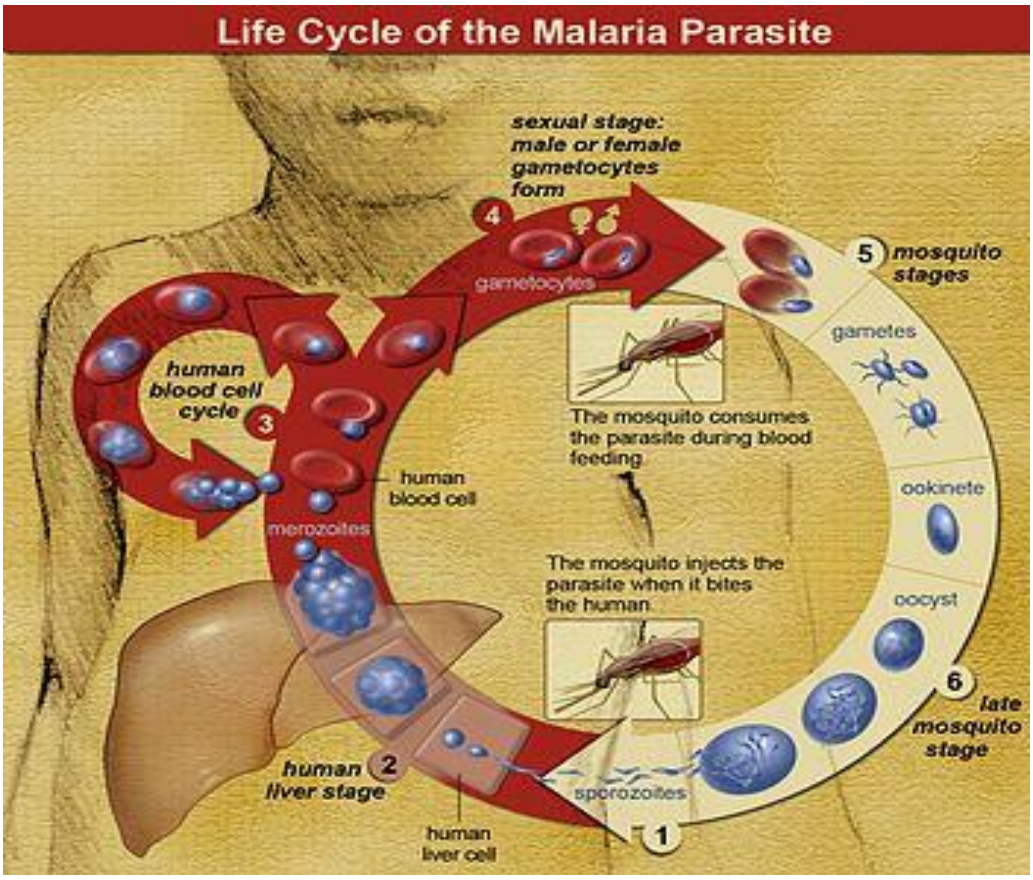


Figure 2: Pictorial representation of life cycle of malaria parasite

Source: National Institute of Allergy and Infectious Diseases (NIAID)

2.7 Factors Associated with Malaria Parasitaemia among Children less than Five Years

Variations in environmental or human related factors can have dramatic consequences for malaria transmission due to the low immune status of the human population especially children.[99] Different factors can drive these changes by influencing the vector's transmission capacity and the malaria prevalence. These factors can be grouped into three classes: (1) environmental factors such as altitude and climate (2) biological factors related to the *Anopheles* vector, the parasite and the human host and (3) human related factors such as socio-economic status, access to health facilities, migration, gender, control activities (IRS, Insecticide Treated Net, and Intermittent Preventive Treatment, use of prophylasis)[100] and land use (irrigation, deforestation, swamp drainage and living near breeding sites). Due to these factors several studies have been conducted aimed at assessing control activities. In a study among under fives attending Primary Healthcare Clinics in Makurdi, Nigeria Jombo *et al* [101] reported a 25% rate of ownership and usage of insecticide treated bed net. Though in the nationwide survey on household ownership and usage of net 23.9% of household own bed nets, while 10.1% own Insecticide treated net. With respect to usage 11.5% use any net while only 1.7% use ITN.[102]

In view of the vital role ITNs play in the actualization of the Roll Back Malaria initiative, Jombo [101] opinioned that these rates of usage were very low. Another study reported 47.9% prevalence of malaria parasitaemia among children that used insecticide treated net as a method to prevent mosquito bites and 90.9% prevalence among those not using insecticide treated net.[90] HIV/AIDS and malaria are both disease of the poor and contribute to the poverty of sub-Saharan African nations by taking a heavy toll on young people who contribute greatly to the workforce of the local economy.[103] Teklehaimanot[103]

opinioned that a strong correlation exist between malaria and poverty because it impedes economic growth and keeps households in poverty, hence the burden of the disease is higher among the poor living in rural setting. This is because they are more likely to be ignorant of preventive measures and their children poorly nourished. Stagnant pools and poor environmental conditions, encourages the breeding of mosquitoes, consequently mosquito vector are more abundant in the rural setting because of the usually unkempt environment, thereby increasing the intensity of transmission.[103] Olasehinde *et al*.[90] in their study observed malaria prevalence rates of 52.2% among children whose parents lived in a clean and hygienic environment compared with children whose parents live in a hygiene compromised environment a prevalence of 74.1%.[90] Studies from other parts of Africa on malaria preventive measures have shown that proper mosquito net use reduces malaria incidence among children by anywhere from 14 to 63 per cent. [104] With ITN use, all cause-mortality in children has been shown to decline by 25 per cent in The Gambia,[105] 33 percent in Kenya[106] and 17 percent in Ghana.[107] Based on findings such as these, the promotion of ITN use has become a central element of national and international efforts against malaria.[108] The United Nations Children's Funds (UNICEF)[8] corroborates that under-five mortality rates could be reduced by about 25-30% if all young children in malaria endemic areas were protected by treated bednets at night. Important components according to the World Health Organization for reducing the burden of malaria morbidity and mortality include more sensitive diagnostic tools; effective use of antimalarial drugs, and improved personal protection and mosquito control.[109] The approach to elimination or control of malaria includes these basics, along with improvements in tracking of human illness and parasite surveillance, and effective

resource delivery. Fortunately, effective interventions for preventing malaria mortality in children are available and are being scaled-up across malaria endemic areas of Africa. These include insecticide-treated mosquito nets (ITNs), indoor-residual spraying (IRS) and intermittent preventive therapy in pregnant women (IPTp). Both ITNs and IRS prevent malaria transmission by reducing the chances that an individual will be bitten by an infective *Anopheles* mosquito. ITNs and IRS are recommended strategies for preventing malaria in children with their impact on all-cause child mortality well demonstrated.[104,109]

2.8 Symptoms

The initial manifestation of malaria common to all species are similar to flu-like symptoms and can resemble other conditions such as septicemia and systemic viral illness. This includes headache, fever, fatigue, abdominal discomfort, muscle and joint aches, shivering, vomiting, anorexia, hemolytic anemia, jaundice and convulsions.[110] The classical symptoms of malaria is paroxysm. This is a cyclical occurrence of sudden coldness followed by rigor, and then fever and sweating, occurring every two days (tertian fever) in *P. vivax* and *ovale* infections, and every three days (quartan fever) for *P. malariae*. *Plasmodium falciparum* infection can cause recurrent fever every 36-48 hours or less pronounced and almost continuous fever.[111] In study conducted in Turbo, Columbia among mothers of under five children on perception and response to febrile illness, majority (82%) of the mothers recognized fever, chills, vomiting and weakness as the most frequent symptoms of malaria in the region.[112]

2.9 Diagnosis

As part of the effective management of malaria, prompt and accurate diagnosis is required. Different diagnostic approach commonly use includes; diagnosis based on symptoms and signs of the disease, microscopic diagnosis which is considered as the gold standard, molecular diagnosis, serology and the use of rapid malaria diagnostic kits. The merits and demerits of these methods are well documented.[113,114,115] For example the presumptive approach based on clinical symptoms has resulted in over diagnosis of malaria and mismanagement of non-malaria fever, which results in unnecessary expenditure, incorrect use of antimalarial drugs with increased risk of drug resistance spreading and a delay in establishing the correct diagnosis and treatment of patients.[23,116] Despite its widespread usage, microscopy diagnosis has its inherent challenges. It is time consuming and labor intensive since its requires equipment and well trained and skilled microscopist to examine the blood film and levels of the parasite in the blood consequently microscopic diagnosis is difficult to achieve in rural settings. Secondly, sensitivity of blood films ranges from 75-90% in optimum conditions, to as low as 50%. Molecular diagnosis is expensive and requires specialized laboratory, while serological diagnosis does not detect the on-going infection rather measures memory of past experience.[117]

2.10 Treatment

Antimalarial medications are used for the treatment of malaria infection. World Health Organization recommends the use of artemisinins in combination with other antimalarial i.e. artemisinin combination therapy (ACT) for the treatment of *plasmodium falciparum.*[118] The other antimalarial includes amodiaquine, lumefantrine, mefloqiune or

sulfadoxine pyrimethamine. Howitt *et al* [119] reported 90% effectiveness in the use of ACT for the treatment of uncomplicated malaria. Chloroquine or ACT have been recommended for the treatment of *P.vivax, P.ovale and P.malariae.*[119]

2.11 Caregivers' Knowledge on Fever, Malaria Infection and Management

A number of studies have investigated differences in knowledge and reported health seeking behavior between men and women. Most found either no difference or women who are mostly the caregivers had limited decision-making and financial power to act.[120] This was associated with failures and delays in seeking treatment, with differential understanding of malaria between men and women, and differential health-seeking behaviour.[120] These differences are critical when considering the main child-caring role of women and children's increased vulnerability to malaria. In 2010 the malaria indicator survey reported that 82% of women interviews during the survey knew that malaria is caused by mosquito while 27% attributed malaria to dirty environment, 12% say malaria is caused by presence of stagnant water.[7] Among 200 mothers/caregivers of under five children interviewed in Ijegemo village, Ogun State, Nigeria,[121] 40% of these respondents did not know the exact cause of malaria, 20% attributed it to sunlight, 16% to mosquito, while 85.5% of his respondents identify stagnant water as breeding site of mosquitoes and children 24-36 months old had the highest percentage (65.5%) of *Plasmodium falciparum* infection. The health seeking behavior of caregivers of children under five years differ significantly depending on the level of education of the caregiver and the availability and easy accessibility of health facilities especially in rural areas.[122] In a community based intervention study to assess the impact of health education and home treatment and prevention of malaria among 150 caregivers of under five children in

Jengre, North Central Nigeria,[123] the researchers reported that 40.6% of respondents had adequate knowledge concerning malaria causation, transmission and treatment, 68% of the caregivers/mothers of these children seek help from health facility within 8 hours of onset of fever in the child, while 56% of the respondents treat their children at home. This study concluded that a significant relationship exist between years of formal education of the caregiver and first line treatment option. Similarly in a study conducted among 500 mothers and caregivers of children under five years in an urban slum in Jos, Nigeria, [124] a low level of knowledge of malaria among the study population (49%) was found, only 24.9% of respondents attributed malaria to mosquito bite. The study further reveals that 66% of the respondents will use an approved health facility/clinic to seek help for treatment of malaria in the children, although 92.4% have not heard of Artmisinin Combination Therapy (ACT). Thus the caregiver's knowledge of malaria was influenced by cost of treatment and level of education. In a study to assess malaria related knowledge, attitude and practices among primary caregivers of children 6 months to 5 years living in Kampala, Uganda, 307 caregivers of the children were interviewed, 90% reported mosquito bite as the cause of malaria and fever. Eighty-two percent of women know that malaria is caused by mosquitoes, while 27% say malaria is caused by dirty surroundings, and 12 % say malaria is caused by the presence of stagnant water. Six percent (6%) of women say that eating certain foods causes malaria, and 8 percent of women responded that they did not know what causes malaria.[125]

With an estimated 300-500 million cases of malaria occurring in Africa, and 90% of these cases been children under 5 years of age,[8] a systematic review of 39 qualitative studies that examines beliefs and practices concerning malaria in sub-Saharan African

countries. The reviewers observed that major barriers to prevention reported included a lack of understanding of the cause and transmission of malaria (29/37), the belief that malaria cannot be prevented (7/39), and the use of ineffective prevention measures (12/39).[126] Adequate knowledge of malaria symptoms and treatment among caregivers of children less than five is essential in the management of malaria infection in these children. [127]A study to compare awareness and treatment knowledge of malaria among 1472 caregivers of children less than five years in rural and urban areas of Ado-Odo/Ota LGA of Ogun State, South West Nigeria, reported that 65% respondents attributed cause of malaria to mosquito bite, while 53% will visit a patent medicine store as first step to seeking treatment. Sixty percent (60%) were aware of the use of insecticide treated net as a method for malaria prevention.[128] In another study conducted in South Western Ethiopia to determine the level of knowledge of caregivers of children under the age of five on childhood malaria, 70% of the respondents mentioned mosquito bite as the cause of malaria in children, while others believed that malaria is caused by non biomedical causes such as drinking dirty water, being in the rain and exposure to sun (60%). In response to questions on symptoms of malaria 86% mentioned fever as the commonest symptoms of malaria in children. The three most important malaria preventive measures reported from this study include; clean surrounding (43.7%); personal hygiene (35.7%) and use of mosquito nets (35%).Eighty two percent of the caregivers will seek proper treatment for the children after two days of onset of malaria symptoms.[129]

As part of the global strategies for malaria control is the integrated vector management which includes the use of insecticide treated nets, indoor residual spraying and environmental management. [130] In a study to identify factors affecting utilization of

insecticide treated net among under five children in Makueni district in Kenya (2009), 400 caregivers of the children were interviewed. Majority (88.5%) of the caregivers were aware of insecticide net. Fifty two percent (52.2%) of respondents reported use of insecticide net by their under five children (U5). Important reason reported from this study for non usage of insecticide nets by U5 was non availability of insecticide treated net in the household (72.3%).[131] In another community based study conducted in Ethiopia in 2009, among women with children under the age of five, >90% of the women recognized mosquito bite as the main cause of malaria. Most mothers were familiar with the symptoms of malaria while only 5.6 % of the respondents possessed one or two insecticide treated nets.[132] A similar study conducted among 232 mothers/caregivers of under five children in Kuje Area Council of FCT, Abuja, Nigeria in 2013 to assess knowledge of malaria and prevention practices, Ashikeni reported that only 1.8% and 90.2% of respondents had good and fair knowledge of cause of malaria in the intervention group. Good knowledge of preventive measures was also poor (5.4%) among the women/caregivers. The researchers concluded that mothers of under five children in Kuje had poor knowledge of the cause of malaria and its prevention method and were not using the Government recommended drugs (ACT) for the treatment of uncomplicated malaria.[133]

2.12 Malaria and Anaemia in Children

Anaemia (haemoglobin level < 11 g/dL) remains one of the most important public health problems among children in malaria-endemic countries of Africa. It affects more than half of all pregnant women and children less than five years old and has serious consequences because severe anaemia (haemoglobin level < 5 g/dL) is associated with an

increased risk of death in children.[134-135] Malaria causes anaemia through hemolysis and increased splenic clearance of infected and uninfected red blood cells and cytokine-induced dyserythropoeisis.[136-137] A single overwhelming episode of malaria, or repeated episodes due to reinfection or failure to adequately clear parasitaemia as a result of antimalarial drug resistance may result in life-threatening anaemia, metabolic acidosis, and, if untreated, death. Severe anaemia probably accounts for more than half of all childhood deaths from malaria in Africa with case fatality rates in hospitals between 8% and 18%[138-140] A 12% prevalence of anaemia was reported among 120 children from Kenya who had malaria parasitaemia.[141]

2.13 Complications of Malaria Caused by *Plasmodium falciparum*

Plasmodium falciparum causes *falciparum* malaria, formerly known as subtertian or malignant tertian malaria.[142] It is the most wide spread accounting for up to 90% of malaria cases in Nigeria.[93] *P.falciparum* is the most pathogenic of the human malaria species with untreated infections causing severe disease and death, particularly in young children, pregnant women and non immune adults.[143] The pathogenicity of *P. falciparum* is mainly due to cycloadherence of *falciparum* parasitized red cells, causing the cells to adhere to one another and to the walls of capillaries in the brain, muscles, kidneys and elsewhere and in pregnant women, in the placenta. Sequestration of parasitized cells in the microcirculation causes congestion, hypoxia, blockage and rupturing of small blood vessels. High levels of parasitaemia resulting in the activation of cytokines and the destruction of many red cells.[144] *Falciparum* malaria parasitaemia can exceed more than 250,000 parasites/µl of blood. Up to 30-40% of red cells may become parasitized. Severe

falciparum malaria is associated cerebral malaria, hemoglobinuria, severe anaemia, hypoglycaemia, and complications in pregnancy have been reported.[145]

2.14 Direct and Indirect Cost of Malaria

Malaria has significant measurable direct and indirect costs, and has recently been shown to be a major constraint to economic development. The direct costs of malaria include a combination of personal and public expenditures on both prevention and treatment of the disease. Personal expenditures include individual or family spending on insecticide treated mosquito nets (ITNs), doctors' fees, anti-malarial drugs, transport to health facilities, support for the patient and sometimes an accompanying family member during hospital stays. Public expenditures include spending by government on maintaining health facilities and health care infrastructure, management and vector control, education and research.[146] In some countries with a heavy malaria burden, the disease may account for as much as 40% of public health expenditure. The indirect costs of malaria include lost productivity or income associated with illness or death. This might be expressed as the cost of lost workdays or absenteeism from formal employment and the value of unpaid work done in the home by both men and women. In the case of death, the indirect cost includes the discounted future lifetime earnings of those who die. [69,147]

2.15 Malaria Control Strategies

Malaria is an entirely preventable and treatable mosquito borne infection. In 2009 the National Malaria Control Programme developed a strategic plan (2009-2013) with the goal of reducing malaria related mortality in Nigeria by 50% by the end of 2013.[6] To achieve this goal the following targets were projected.

1. At least 80% of households have two or more ITNs

2. At least 80% of pregnant women and children under five sleep under an ITN
3. Twenty percent of households nationwide are covered by IRS as a complementary strategy to ITNs, and where conducted, at least 85% of targeted structures are adequately sprayed
4. At least 80% of pregnant women receive two doses of IPTp
5. At least 80% of patients with fever attending a health facility receive an appropriate malaria diagnostic test, and those testing positive are effectively treated according to the national treatment guidelines.

Though widespread, there has been some evidence of low coverage of ITNs use.[103] In Nigeria millions of pregnant women are now also provided with prophylactic anti-malaria drugs as part of the Interruptive Preventive Treatment (IPT), during their second and third trimesters to prevent infections.[148]

Malaria control and interventions have been implemented and in the recent past intensified as an effort to attain the World Health Assembly, Roll Back Malaria, and Millennium Development Universal targets with the aim of reducing and interrupting disease transmission in sub Saharan Africa. Jos is a malaria endemic area in which malaria control measures such as the use of Artemisinin Combined Therapy (ACT), the use of insecticide treated bed nets (ITNs), and Intermittent Preventive Treatment (IPTp) for pregnant women and children have been implemented. Inspite of these efforts the overall prevalence of malaria infection from studies recently conducted in Jos remains high among the under-five children: (38-56%).[34-36] This suggest that there are factors that are responsible for the high prevalence and thus it is very important that research be conducted to identify the gaps. Therefore this work is necessary to confirm the

prevalence and circulating species of malaria parasitaemia as well as assessing the factors (socio-demographic, environmental,) associated with malaria parasitaemia among HIV positive and HIV negative under five children attending selected health facilities in Jos, North Central Nigeria.

CHAPTER THREE – METHODOLOGY

3.1 Study Area

This study was conducted in Jos North Local Government Area of Plateau State from Mid October, 2013 to February, 2014. Jos is located at latitude 9° 55' to 10°N and longitude 8°52' to 9°E) in the middle belt, North Central of Nigeria. The city has a population of 900,000 residents based on the 2006 census, with an altitude of 4,062 feet (1,217m) above sea level. It enjoys a more temperate climate than much of the rest of Nigeria (average monthly temperature range from 70^0 to 77^0F or 21^oC to 25^oC); from mid Nov to late January, night time temperatures drop as low as 11 degree Celsius resulting in chilly nights. Jos city receives 1,400 millimeters (55.1 in) of rain fall annually, which peaked during the wet season months of July and August. The average lower temperatures in Plateau State as a whole has lead to a reduce incident of malaria infection though transmission is usually intense as the end of the rainy season approaches.[149] The prevalence of HIV infection in the state is 4.88%.[150] The U.S President's Emergency Plan for AIDS Relief (PEPFAR) program currently supported sites situated within health facilities in Jos metropolis and the General Peadiatric Out Patient Department were used for this study namely; Jos University Teaching Hospital (JUTH), Plateau Specialist Hospital, Bingham University Teaching Hospital, Our Lady of Apostle Hospital (OLA) and Faith Alive Foundation.

3.2 Study Design

A comparative cross-sectional study was conducted among children less than 5 years old living with HIV and those without HIV.

3.3 Study Population

The study population were children between 6 months and 59 months old attending selected health facilities.

3.3.1 Inclusion Criteria

Children aged 6 months to 59 months living with HIV, attending the ART clinic in the selected health facility whose parent gave consent were recruited for the study.

All Children aged 6 months to 59 months visiting the General Outpatient Department of the selected health facilities whose parent consent were recruited for comparison.

3.3.2 Exclusion Criteria

Children aged 6 months to 59 months living with HIV who were too sick or on admission on account of other opportunistic infections or those whose parents did not consent were excluded from the study.

3.4 Sample Size Determination

The minimum sample size required for this study was calculated based on the documented malaria prevalence of 38% [34] among HIV negative under five children in Jos, while the proportion of exposure to malaria parasitaemia among children less than 5 years old living with HIV was assumed to be 50%. The formula for comparison of two proportions [151] was used to compute the sample size as follow:-

$$n \text{ (each group)} = \frac{(P_1 q_1 + P_2 q_2)(Z_{1-\alpha/2} + Z_{1-\beta})^2}{(P_1 - P_2)^2}$$

Where

P_1 = the prevalence of malaria among under five children without HIV

P_2 = the assumed prevalence of malaria among under five children living with HIV

$q_1 = 1 - P_1$

$q_2 = 1 - P_2$

$Z_{1-\alpha/2}$ = value of the standard normal distribution corresponding to a significance level of alpha (i.e., 1.96 for two-sided test at the 0.05 level)

$Z_{1-\beta}$ = value of the standard normal distribution corresponding to the desired level of power (i.e., 0.84 for a power of 80%)

$$n = \frac{(0.38 \times 0.62) + (0.5 \times 0.5)(1.96+0.84)^2}{(0.5-0.38)^2}$$

n = **264** (in each group)

3.5 Sampling Technique

Systematic random sampling

The total number of under five (U5) children living with HIV attending the ART clinic in the selected health facility (HF) was obtained from the register. This formed the sample frame. The number of the children sampled was done by proportionate sample allocation. The total number of children less than five years in each of the ART clinics was multiplied by the sample size calculated and divided by the total population of children less than five years obtained from all the health facility. The sampling interval was

obtained by dividing the total study population by the calculated sample size. The first child was selected by simple random method by balloting.

Sample size calculated = 264

Total number of U5 registered from all the HF = 612

Total U5 registered with JUTH = 222

= 222 × (264 ÷ 612) = **96**

Sample interval = 612÷264 = 2.2

The same process was done for the other health facilities used until the allocated sample size was reached .The table below shows the total number of under five children living with HIV registered with the antiretroviral (ART) clinic in each of the selected health facility in Jos and the sample size obtained from each.

Table 3.1: Number of children less than five years registered with the ART clinic in each health facility and sample size allocated

Health facility (ART Clinic)	Total under 5 registered	Sample size allocated
Jos University Teaching Hospital	222	96
Plateau Specialist Hospital	171	73
Our Lady of Apostle Hospital	74	32
Bingham University Teaching Hospital	62	27
Faith Alive Foundation	83	36
Total	612	264

At the Pediatric Out Patient Department of each of the health facilities the average weekly attendance of under five children was obtained. The figure was multiplied by 4 to get the monthly average. The monthly average was multiplied by the number of months used for data collection which was 5 months. The sum total from all the health facilities now serves as the sample frame. Table 2 below gives the details. This was done on every clinic day from mid October, 2013 to February, 2014 until the required sample size was attained.

To ensure that no patient was recruited twice the hospital number of each child screened both at the ART clinic and the Pediatric General Outpatient Department of each of the selected health facilities were recorded on the consent form and kept securely.

Table 3.2: The average weekly and monthly attendance of HIV negative under fives and the sample size allocated from each Health Facility

Health Facility	Weekly average of U5 attendance	Monthly average (×4)	Sample frame (×5)	Sample size allocated
JUTH	15	60	300	81
P.S.H	10	40	200	54
OLA Hospital	7	28	140	38
B.U.T.H	9	36	180	48
F.A.F	8	32	160	43
Total	49	196	980	264

3.6 Study Instrument

A structured interviewer administered questionnaire (adapted from previous study) [89] was used to obtain information on age, sex and other socio-demographic attributes, which includes; occupation, level of education, income level of caregivers. Knowledge of mothers/caregiver on fever and malaria infection, malaria prevention practices, potential risk factors associated with malaria infection, history of previous malaria diagnosis, intake of anti- malarial drugs in last 2 weeks and use of cotrimoxazole prophylaxis. The questionnaire was pretested at Mandela clinic, Vom which is a setting similar to the study sites. All needed modifications were made on the questionnaire based on the responses from the respondents before being administered at the study site.

3.7 Data Collection Methods

Two research assistants were trained from each of the selected health facilities on universal precautions, good clinical and laboratory practice, quality control and quality assurance, safety and maintenance of laboratory-related documents. Also training was

done on preparation of thick and thin blood films, staining procedure and data collection techniques using the structured questionnaire.

3.7.1 Laboratory testing and specimen source

Three (3) mls of venous blood was collected aseptically from each enrolled child into Potassium (K3) ethylene diamine Tetra -Acetate anticoagulant bottles. The blood was used for the assessment of thin and thick films for malaria parasitaemia, haemoglobin estimation and Cluster of Differential-4 (CD4+ T-cell) percent enumeration.

3.7.2 Haemoglobin estimation

The blood was mixed for approximately 5 minutes using the Dynal sample mixer. This was imputed into the Mindray three parts differential auto analyzer. Twenty (20) µl of the whole blood was aspirated and analyzed within 2 minutes. The result was displayed on the LCD screen and printed out. Anemia was defined as Hb < 11g/dl. The normal range for haemoglobin for U5 is 11-13g/dl.[152]

3.7.3 Determination of CD4+ T-cell percentage

Ten (10 µl) of CD4% CD4 monoclonal antibody (mAb) was added to a clean test tube. To this 10 µl of CD4% CD45 mAb was added and 20 µl of the whole blood was also added. This was mixed and incubated at room temperature for 15 minutes in the dark. Then 400 µl of CD4% easy count buffer 1 was added and gently mixed. Just before sample run 400 µl of CD4 easy count buffer 2 was added and mixed gently. The preparation was analyzed using the PARTEC cyflow machine version 1.9. The CD4 % results were read from the screen.

3.7.4 Detection of malaria parasite

An immunochromatograpy RDT cassette (CareStart^{TM}) for *Plasmodium falciparum* that detects histidine-rich protein 2 (HRP2) antigen in the blood was performed following the manufactures instruction. Briefly described, 5 µl of blood was dropped into the sample well in the test device, two drops (60µl) of assay buffer was added into the assay buffer well. The test result was read in 20 minutes. The presence of two colour bands (i.e. the test and the control) indicates a positive result, while the presence of only one band within the result window indicates a negative result. The test was considered invalid if the control line does not appear. To carry out the malaria microscopy test, a drop of blood each was placed on 2 clean grease free glass slides for thin and thick blood film. The slides were made in duplicates and appropriately labeled. Thin and thick blood smears were prepared according to World Health Organization (WHO) guideline for malaria microscopy.[153] The thin film was fixed using methanol for about two seconds and both films were left to air dry for 24 hours and then stained with 3% quality controlled Giemsa stain at P^H 7.2 for 45 minutes as recommended by WHO.[153] Also phosphate buffer at P^H 7.2 were used to rinse the stained slides. Blood films were examined microscopically using 100 magnification (Oil immersion) lens as described by Cheesbrough.[154] At least 100 microscopic fields were examined and 200 WBC counted. The thin blood film was used for species identification. The total parasite counts were recorded as number of parasites per microlitre of blood. The Parasite density per microlitre of blood was estimated from the thick film, taking the putative mean number of Leucocytes per microlitre of blood as 8,000 expressed as

$$\text{Parasite density} = \frac{\text{parasite count} \times 8000}{\text{Number of WBC counted}}$$

Malarial parasitaemia was defined as the presence of at least one asexual parasite in the blood film of the study participant.

A blood smear was declared negative if no asexual parasite were seen after examining 100 high power microscopic fields and no asexual parasites were found.

Fever was defined as axillary body temperature $\geq 37.5^{0}$C and was measured using digital clinical thermometer.

3.7.5 Quality control and quality assurance procedures

To ensure accuracy of test result two experienced laboratory scientist examined the slides. In the event of discordant results, the slides were re-read again and where the differences in the mean parasite counts of the two microscopist were between 10-20%, the result was accepted otherwise a third microscopist independently examined the slides serving as a tie breaker. Furthermore 10% of the positive and negative slides were selected randomly and transported to Ahmadu Bello University Hospital, Shika in Zaria where a WHO certified microscopist re-examined the slides. For the CD4 T cell percent, the count check beads of known concentration was run every time to validate assay runs. Values obtained were plotted on a levy-Jennings chart.

3.8 Data Management

All data were entered into the Epi info statistical software version 7 (Epi Info™ 7)

For descriptive data, percentage was used to assess the outcome of interest. Proportions were compared using chi-square test or Fisher exact test (univariate analysis); at a significant level of 0.05. Bivariate analysis was done to determine association between HIV status, other risk factors and levels of parasitaemia.

3.8.1 Grading of knowledge

Knowledge of the participants was scored on a five-point domain to assess their level of knowledge. For each question asked, one point was scored for correct response and zero for wrong response; thus, the maximum score was five and the minimum score was zero. Anyone who scores 2 points and below, was graded as having poor knowledge, while scores of 3 points and above was graded as having good knowledge.[155] The questions that were asked to grade the level of knowledge were all open-ended questions so as to reduce the possibility of guessing.

The first question asked was on the general causes of fever in children. The only correct response as stated in the questionnaire was possible bacteria infection. The second question asked was on how children get malaria fever. The correct response was when bitten by mosquito. The third question asked was on symptoms of malaria of which the correct response was fever, chills and rigor, flu-like illness, vomiting and poor appetite. The forth question centered on assessing knowledge of the caregivers on the first ideal steps to take whenever a child presents with malaria fever. The correct response was to tepid sponge the child, administer simple antipyretic and go to the nearest clinic or hospital. The last question was on knowledge on the type of drug use for treatment of malaria fever. The correct response was antimalarial (e.g. ACTs, CQ, SP,)

3.8.2 Statistical analyses

Bivariate analysis was done to determine if there is an association between levels of parasitaemia and HIV status using Chi-square test at 5% significant level. Also an association between parasitaemia and use or non usage of insecticide treated bed nets, environmental conditions, antimalarial intake in the past 2 weeks and use of

cotrimozaxole prophylaxis was determined. Logistic regression analysis was done and adjusted Odds ratio was computed to determine the relationship between CD4 counts (%), Hb levels and parasitaemia.

3.9 Ethical Consideration

Ethical approval was obtained from Jos University Teaching Hospital (JUTH) ethical committee, Bingham University Teaching Hospital Review Board, Plateau State Specialist Hospital Health Research Ethics Committee and Faith Alive Foundation Ethics committee. Informed consent was obtained from parent/caregiver of the participants. To maintain confidentiality participants name only appeared on the consent form, but not the questionnaire and all records were kept securely.

3.10 Limitations

This is a hospital based study and hence cannot be generalized for the entire population. Information provided by the caregivers on presence or absence of perceived risk factors were not verified. Though efforts were made to check the child's hospital file to verify clinical information.

CHAPTER FOUR - RESULTS

Table 4.1: Age and sex distribution of under five HIV positive and HIV negative children attending health facilities in Jos, North Central, Nigeria 2014

Variable	HIV positive		HIV negative	
	Freq (n=264)	Proportion (%)	Freq (n=264)	Proportion (%)
Sex				
Male	153	58	113	43
Female	111	42	151	57
Age (months)				
<12	04	1.5	21	8
12-23	23	8.7	78	30
24-35	65	24.6	74	28
36-47	67	25.4	51	19
48-59	105	40	40	15

Five hundred and twenty eight (528) children less than five years were enrolled in all, of which 264 were living with HIV and were on antiretroviral therapy and the other half were negative for HIV. The response rate was 100% as all the caregivers consented.

The mean age of children less than five years living with HIV was 40.6 ±13.7 months while the mean age of children less than five years without HIV was 28.7 ±14.2 months. Among children living with HIV, 111(42%) were females while among those without HIV 151 (57%) were males. The age and sex distribution of the children living with HIV and those not living with HIV is presented in table 4.1.

Table 4.2: Distribution of Malaria Parasitaemia by Sex and Age Group of Children less than 5 Years Attending Health Facilities in Jos, North Central, Nigeria 2014

Characteristics	HIV positive		HIV negative	
	M. P positive (n=42)	M. P negative (n=222)	M. P positive (n=61)	M. P negative (n=203)
Sex				
Females	17 (15.3)	94 (84.7)	21 (18.6)	92 (81.4)
Males	25 (16.4)	128 (83.6)	40 (26.5)	111 (73.5)
Age (months)				
<12	01(2.4)	3 (1.4)	03 (4.9)	18 (8.9)
12-23	06 (14.3)	17 (7.7)	16 (26.2)	62 (30.5)
24-35	02 (4.8)	11 (4.9)	11 (18.1)	22 (10.8)
36-47	15 (35.7)	104 (46.8)	18 (29.5)	74 (36.5
48-59	18 (42.8)	87 (39.2)	13 (21.3)	27 (13.3)

MP = Malaria parasitaemia

Of the 264 under five children living with HIV, 42 had thick blood smear positive for parasitaemia, hence the prevalence of malaria parasitaemia among them was 16%. Among under five HIV negative children, 61 had positive thick smear with a prevalence of 23.11%. Among children less than 5 years living with HIV who had malaria parasitaemia, 17 (15.31%) were females, while among under five HIV negative children who had malaria parasitaemia 40 (26.5%) were males. The highest level of parasiteamia 18 (42.9%) among children living with HIV was found in older children 48-59 months, while among HIV negative children aged 24-35 months had greater level of malaria parasitaemia,19 (31%).

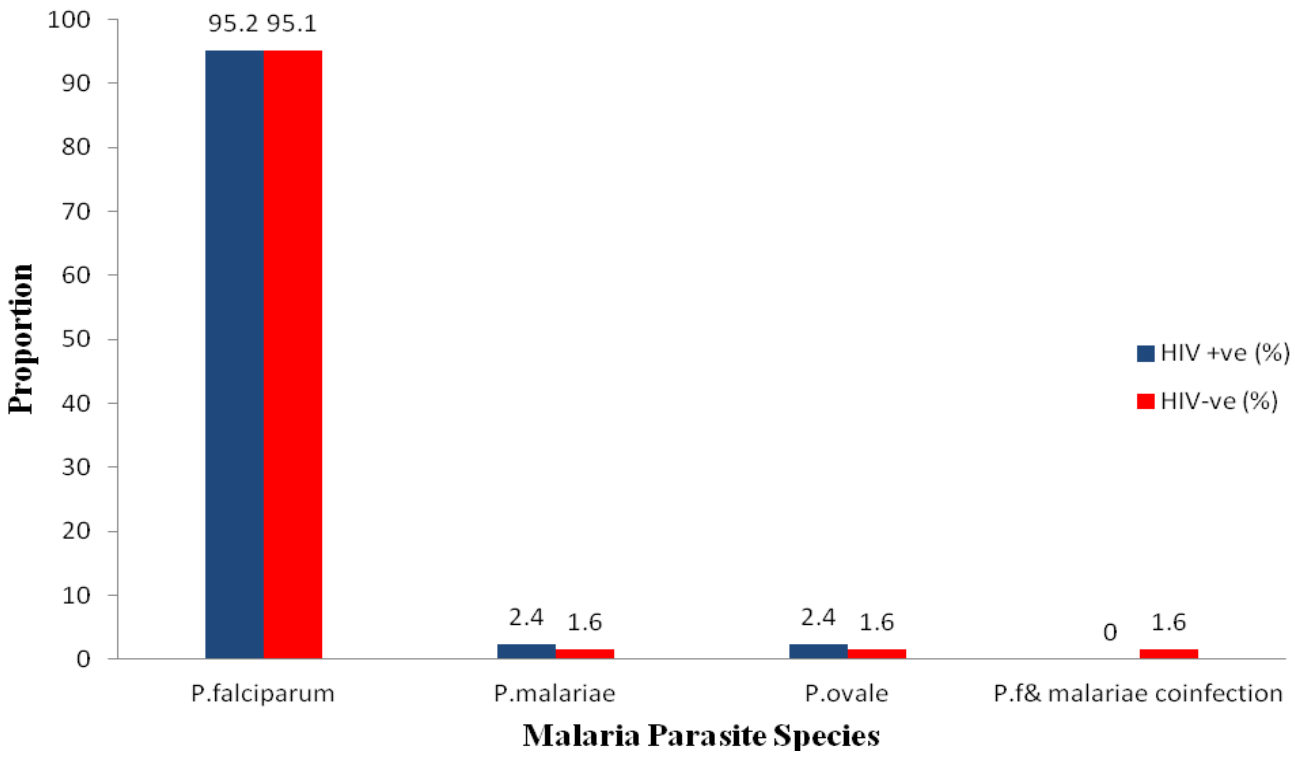


Figure 3: Species of Malaria Parasite found among Children less than 5 years in Health Facilities in Jos, 2014

The thin blood smear revealed that the predominant specie found among children with HIV was *Plasmodium falciparum,* 40 (95.2%) However, one thin smear had *plasmodium malariae* and another *ovale.* Among under five children living with HIV with positive smears only 6 (14.3%) were detected using the Histidine rich protein 2 antigen for *Plasmodium falciparum* (a rapid malaria diagnostic test kit). While among HIV negative children, 8.2% were detected by the RDT. *Plasmodium falciparum* was found in 58 (95.2%) of children who are HIV negative. A co infection of *plasmodium falciparum* and *malariae* was also observed in one slide.

Table 4.3: Distribution of Parasite Density with Respect to Sex and Age group of Children less than 5 years attending Health Facilities in Jos, 2014

Variable	Distribution: parasite densities (parasites/µl)			
	<1000		1000-5000	
	HIV positive (%)	HIV negative (%)	HIV positive (%)	HIV negative (%)
Sex				
Male	9 (36)	10 (25)	16 (64)	30 (75)
Female	7 (46.6)	6 (28.6)	9 (55.2)	15 (71.4)
Age (months)				
<12	1 (6.3)	1 (6.3)	0 (0.0)	2 (4.4)
12-23	3 (18.8)	3 (18.8)	3 (12.0)	13 (28.9)
24-35	0 (0.0)	2 (12.5)	2 (8.0)	9 (20.0)
36-47	6 (37.5)	8 (50)	8 (32.0)	10 (22.2)
48-59	6 (37.5)	2 (12.5)	12 (48.0)	11 (24.4)

Mean density HIV positive = 1475 parasite/µl; HIV negative =1632 parasite/µl

Thirty (75%) of male children less than 5 years who are HIV negative had parasite density between 1000-5000 parasite/ µl of blood, while among HIV positive male children 16 (64%) had parasite density between 1000-5000 parasite/ µl of blood. The least parasite density found among HIV negative females was 6 (28.6%). The age group 36-59 months had greater parasite density (1000-5000 parasite/µl) among under five children living with HIV. The malaria parasite density was low among children less than 12 months in the 2 study population. The mean parasite density among children living with HIV was 1475 parasite/µl, while the mean parasite density among children who are HIV negative was 1632 parasite/µl.

Table 4.4: Distribution of Malaria Preventive Methods used by Caregivers of HIV Positive and HIV Negative Children in Health Facilities in Jos, 2014

Preventive method	HIV positive (n=264)		HIV negative (n=264)	
	Frequency	Proportion (%)	Frequency	Proportion (%)
ITN ownership				
Own at least 1 net	76	28.8	133	50.4
More than 1 net	153	57.9	82	31.1
Do not have net	36	13.3	49	18.5
ITN usage				
Use ITN	201	76.1	195	73.8
Never use ITN	63	23.9	69	26.2
Presence of doors & window nettings	171	64.7	195	73.9
Use of insecticide sprays	167	63.3	164	62.1
Use of prophylaxis				
(a) Cotrimozaxole	243	92.1	0	0
(b) Antimalarial intake	50	18.9	24.9	9.1

Fifty eight percent of caregivers of children less than 5 years living with HIV own more than one insecticide treated bednet, while 133 (50%) of caregivers of HIV negative children own at least one insecticide treated mosquito net. Majority of the children living with HIV, 201 (76%) use an insecticide treated net. Among HIV negative children 69 (26%) has never use an insecticide treated bed net. Therefore the most malaria preventive method used by caregivers of children less than 5 yrs living with HIV was insecticide treated nets. Among caregivers' of HIV negative children, 195 (73.9%) had door and window nettings in the house where the child resides. Majority, 243 (92%) of the children living with HIV were taking cotrimozaxole prophylaxis. Only 24 (9%) took antimalarial in the past two weeks prior to the interview among HIV negative children. Twenty two (8.3%) of caregivers of children living with HIV who had no net reported that insecticide treated net is not readily available, while 29 (11%) of caregivers of HIV negative children reported non availability of the net, only 7 (3%) said insecticide treated net is too expensive, while among caregivers of children living with HIV, 5 (2%) said mosquito net is too expensive.

Table 4.5: Relationship between factors associated with malaria parasitaemia and positive malaria parasite result among HIV positive and HIV negative under 5 children in Jos, 2014

Risk factor	HIV positive	HIV negative	Odds ratio	95% CI	χ^2	P value
	Malaria positive (n= 42)	Malaria positive (n= 61)				
Presence of stagnant water						
1.Yes	6	1	9	1.3-85*	4.3	0.01
2. No	36	59				
Presence of refuse dump sites						
1.Yes	9	12	1	0.4-2.8	0.005	1
2. No	33	48				
Stream/river						
1.Yes	9	5	3	0.9-9.1	2.6	0.08
2. No	33	55				
Grasses						
1.Yes	10	5	3	1.4-11*	3.7	0.04
2. No	32	56				
Travel history past 21 days						
1.Yes	5	3	2.6	0.5-11	0.9	0.2
2. No	37	58				
Malaria prevention methods						
ITN ownership						
1.Yes	40	49	4	1-23*	3.5	0.03
2. No	2	12				
Net use						
1.Yes	8	13	0.8	0.3-2.3	0.001	0.8
2. No	34	48				
Insecticide sprays						
1.Yes	26	38	0.9	0.6-1.5	0.03	1
2. No	16	23				
Antimalarial intake						
1.Yes	32	52	0.5	0.2-1.5	0.8	0.3
2. No	10	9				

*statistically significant

Children living with HIV who resides where there are pools of stagnant water were more likely to have malaria parasite detected from their peripheral blood [OR: 9; 95% CI: 1.3-85; χ^2: 4.3; P = 0.01].

The presence of grasses surrounding the vicinity where the child resides was associated with having positive malaria parasite result among children less than five 5 years living with HIV [OR: 3.0; 95% CI: 1.4 -11; χ^2 : 3.7; P = 0.04]. Similarly the presence of stream/river close to the house where children less than five years resides was a risk factor for positive malaria parasitaemia [OR: 3; 95% CI: 0.9-9.1; χ^2: 2.6; P = 0.08]. No association was observed between positive malaria parasiteamia among children living with HIV and the presence of refuse dump site close to the house where these children resides [OR: 1; 95% CI: 0.4-2.8; χ^2: 0.005; P = 1]

Children living with HIV whose caregivers uses insecticide spray as malaria preventive method were less likely to have malaria parasitaemia [OR: 0.9; 95% CI: 0.6-1.5; χ^2 : 0.03; P = 1]. However this was not statistically significant. The intake of antimalarial by children less than 5 years in the previous 2 weeks prior to the interview was protective against malaria parasitaemia [OR: 0.5: 95% CI: 0.2-1.5; χ^2 :0.8; P = 0.3]. Children who use insecticide treated mosquito net (ITNs) were less likely to have positive malaria parasitaemia compared with those who did not use ITNs [0R: 0.8; 95% CI: 0.3-2.3; χ^2: 0.001; P = 0.8].

Table 4.6: Logistic regression of factors associated with malaria parasitaemia and positive malaria parasite result among HIV positive and negative under 5 children in Jos, 2014

Risk factor	Adjusted Odds ratio	95% CI	P value
Presence of stagnant water	7.8	0.7-29.0	0.22
Presence of grasses	3.3	1.3-14.2	0.03*
ITN ownership	3.6	0.7-18.3	0.1

*statistically significant

The only statistically significant risk factor was the presence of grasses in the vicinity of the house where the child resides

Table 4.7: Socio-demographic Characteristics of Caregivers of HIV positive and HIV negative Children under five years of age in Health Facilities in Jos, Nigeria 2014

Characteristics	Caregivers' Response			
	HIV positive		HIV negative	
	Frequency	Proportion (%)	Frequency	Proportion (%)
Sex				
Female	238	90	251	95
Male	26	10	13	5
Marital Status				
Single	17	6.4	24	9.1
Married	237	89.8	223	84.5
Divorced	3	1.1	5	1.9
Widow	7	2.7	12	4.6
Occupation				
Employed	157	59.5	155	58.7
Unemployed	23	8.9	19	34.1
Housewife	84	31.8	90	34.1
Education Level				
At least secondary	169	64	160	60.6
Below secondary	95	36	104	39.4
Income Level				
<₦18,000/month	116	74	113	73.4
>₦18,000/month	41	26	41	26.6

The mean age of caregivers of children less than five years living with HIV and those without HIV receiving care in the selected health facilities were 31.3 years ± 8.5 and 30.2 ± 7.6 respectively. In all 223 (84.47%) of caregivers of children with HIV and 237 (87.77%) of caregivers of HIV negative children were married with 233 (88.3%) and 211 (80%) been mothers of the children respectively. At least 169 (64%) and 160 (60.6%) of the caregivers of HIV positive and negative children had secondary education respectively.

Table 4.8: Caregivers Knowledege on Fever and Malaria Infection among Children under five years of age attending Health Facilites in Jos, 2014

Characteristics	Caregivers' response			
	HIV positive (n=264)		HIV negative (n= 264)	
	Frequency	Proportion (%)	Frequency	Proportion (%)
Caregivers Knowledge on causes of fever in children				
Change in weather	64	24.2	56	21.2
Teething	52	19.7	42	15.9
Drinking dirty water	61	23.1	75	28.4
Natural to have fever	17	6.4	13	4.9
Bacterial infection	70	26.5	78	29.5
Malaria transmission				
Mosquito bite	254	96.2	257	97.4
Malaria symptoms				
Fever	235	89.6	207	78.4
Rigor & chills	56	21	47	17.8
Vomiting	45	17.1	32	12.1
Poor appetitte	39	14.7	40	15.2
Flulike illness	51	19	20	7.6
Malaria management				
Tepid sponge,administer antipyretic, go to clinic	244	81.7	243	91.7
Give any availabe drug	18	6.8	18	6.8
Malaria medication				
antimalarial	148	56.1	191	72.6
Paracetamol	92	35	63	24
Antibiotics	20	7.6	6	3.4

4.8.1 Knowledge on how children get malaria infection

Among caregivers of children less than 5 years (yrs) living with HIV, 254 (96.2%) attributed malaria fever to bite from infected mosquito,while 257 (97.4%) of caregivers of HIV negative children attributed malaria fever to mosquito bites.

4.8.2 Knowledge on symptoms of malaria in children

Two hundred and thirty five (89%) and 207 (78.4%) of caregivers of children less than 5 years living with HIV and caregivers of HIV negative children mentioned fever as one of the symptoms of malaria respectively.Among caregivers of HIV positive children 51 (19%) mentioned flu-like illness while 20 (7.6%) of caregivers of HIV negative children mentioned same.Thirty nine (14.8%) of caregivers of HIV positive children mentioned poor appetite as a symptom of malaria in under 5 children,while 40 (15%) of caregivers of HIV negative children mentioned poor appetite.

4.8.3 Knowledege on first set of steps to take in the management of malaria infection in children

In response to first set of steps to be taken in the management of malaria infection in children less than 5 yrs living with HIV, 244 (81.7%) responded that tepid sponge the child,administer simple antipyretic and go to the nearest clinic or hospital are the first step to take, while 243 (91.7%) of caregivers of HIV negative children reported same. Eighteen (6.8%) of caregivers of children less than 5 yrs living with HIV will administer any available drug to the child as first step to take also 18 (6.8%) of caregivers of HIV negative children said the same thing.

4.8.4 Knowledge on types of drugs appropriate for treatment of child with malaria infection

In response to knowledge of the most appropriate drug to administer to a child with malaria infection,148 (56%) of the caregivers of children less than 5 yrs living with HIV mentioned paracetamol, 92 (35%) mentioned antimalarial (ACTs, CQ,SP), 20 (7.6%) mentioned antibiotics.Among caregivers of HIV negative children, 191 (72.6%) mentioned amtimalarial (ACTs, CQ, SP), while 63 (24%) mentioned paracetamol, only 9 (3.4%) mentioned antibiotics.

4.8.5 Grading of Knowledge of Caregivers on Fever, Malaria Infection and Management

Most, 251 (95%) of the caregivers of children living with HIV had a total knowledge score of four; while 13 (5.0%) had a score of zero. Among caregivers of HIV negative children, 258 (97.7%) had a knowledge score of four. A knowledge score of 3 and above is considered as good knowledge. Therefore among caregivers of children less than 5 yrs living with HIV in the selected health facilities, 251 (95%) had a good knowledge of fever, malaria infection and management, while 13 (4.92%) had poor knowledge. While among caregivers of HIV negative children, 258 (97.7%) had good knowledge of fever, malaria infection and management, while 6 (2.3%) had poor knowledge.

Table 4.9: Haemoglobin Estimation and CD4% among Children less than 5 years in Health Facilities in Jos, 2014

Haemoglobin Estimation (g/dl)	HIV positive		HIV negative	
	Frequency	Proportion (%)	Frequency	Proportion (%)
>8	10	3.8	1	0.4
8-10.9	164	62	124	46.9
11-13	90	34.1	139	52.7
CD4 Percentage	14	5.3		
<15	14	5.3	0.0	0.0
>15	250	94.7	0.0	0.0

CD4% <15 = Antiretroviral therapy initiation point

The mean haemoglobin (Hb) concentration among children less than 5 yrs living with HIV was 10.3 ± 1.2 (5.8-14.9) while the mean haemoglobin concentration among HIV negative children was 11.1 ± 1.2 (7.9-14.7). Among children with HIV, 10 (3.8%) had Hb concentration of <8g/dl, indicating severe anaemia while 164 (62.2%) had Hb between 8-10.9 g/dl indicating mild anaemia, 139 (52.7%) fall within the normal range of 11-13 g/dl for paediatrics. Among under 5 HIV negative children, 1 (0.38%) had Hb concentration of <8g/dl, while 124 (47%) had Hb between 8-10 g/dl indicating mild anaemia, 90 (34.1%) fall within the normal range. The overall prevalence of anaemia among the children living with HIV was 66%, while among HIV negative children was 47.3%. The mean CD4% of children living with HIV was 33.6% ± 14.2%.

Table 4.10: Relationship between Anaemia, CD4 % and presence of Malaria Parasitaemia among Children less than five years in Health Facilities in Jos

Variable	HIV positive children			HIV negative children		
	Proportion with parasitaemia	Odds ratio (95% CI)	P	Proportion with parasitaemia	Odds ratio (95% CI)	P
Anaemia present	32/42 (76.2%)	0.5 (0.3-1.2)	0.2	21/61 (34.4%)	2 (1.7-3.6)*	0.02
No Anaemia	10/42 (23.8%)			40/61 (65.6%)		
CD4 Percent						
< 15%	2/14 (14.3%)	1.1 (0.2-5.3)	1	NA	NA	NA
>15%	40/250 (16%			NA	NA	NA

NA= Not applicable

The presence of anaemia was significantly associated with malaria parasitaemia among HIV negative children. (OR 2; 95% CI 1.7-3.6). The adjusted odds ratio obtained was 1.85 (aOR 1.85; 95% CI 1.4-4.2). The odds of malaria parasitaemia were not statistically associated with anaemia among children living with HIV. Also no association was found between CD4% and presence malaria parasitaemia among children living with HIV.

Table 4.11: Logistic regression of the relationship between Anaemia, CD4 % and presence of Malaria Parasitaemia among Children less than five years in Health Facilities in Jos

Variable	HIV positive children		P value	HIV negative children		P value
	Adjusted Odds ratio	95% CI		Adjusted Odds ratio	95% CI	
Anaemia Present	0.7	0.4-1.6	0.3	1.8	1.4-4.2	0.04*
No anaemia						
CD4 percent						
< 15	0.9	0.1-3.8	1.2	NA	NA	NA
>15						

*significant ; NA= Not applicable

The logistic regression analysis shows that the presence of anaemia among HIV negative under five children was not due to chance, but was truly statistically significant.

CHAPTER FIVE - DISCUSSION

The prevalence of malaria parasitaemia among under five children living with HIV was 16% while among HIV negative children the prevalence was 23.11%. The overall prevalence of malaria parasitaemia among the under five children in this study was 19.5%. This is considerably low and may be attributed to the continuous intake of cotrimoxazole prophylaxis by children living with HIV and the seasonal variations in parasitaemia intensity. The low prevalence of 16% found among under five children living with HIV in this study was similar with findings from Oshogbo, South West Nigeria, where a prevalence of 18.5% was reported among children living with HIV.[90] However Studies conducted among 132 Ugandan children reported 2.3% prevalence of uncomplicated malaria and HIV.[86] This finding contrast with our study. The difference in prevalence may be due to the fact that this study was conducted during an intervention trial evaluating different anti- malarial chemoprevention regimens at a dedicated health facility, hence the low prevalence recorded.

The prevalence of malaria parasitaemia differs from one region to another depending on the endemicity of the disease and the season of the year.[60] This study was conducted during the low transmission season in Jos mid October to February, 2014.This can partly account for the low prevalence recorded. The life cycle of the mosquito and the transmission dynamics of the diseases they transmit have been shown to be strongly influenced by climatic variable.[156] The development of the malaria parasite in the mosquito and rate of biting decreases at low temperature.[149] Also the average lower temperature in Plateau State as a whole had resulted in reduce incidence of malaria infection. This study was conducted during the cold season in Jos. The prevalence of

23.11% found among HIV negative children in this study contrast with findings by Ikeh *et al* [35] who reported a prevalence of 56.9% among under five attending primary health care facility in Jos; Daboer *et al*[34] in 2010 also reported a prevalence of 38% in a community survey of children less than 5 years in Jos metropolis. A recent study reported by Okoli *et al*[36] found a prevalence of 48.06% in a hospital based study of 600 febrile under five children. These studies were conducted during the high transmission seasons, either during the raining seasons or shortly before the end of the rains and the subjects were under five children who were either febrile or presenting with malaria-related symptoms. However in our study children already on antiretroviral and those attending the General Out Patient Department of the health facilities with or without fever and asymptomatic were screened for the presence of malaria parasitaemia during the low transmission season. This partly explains the difference in the prevalence recorded. Other studies from Africa have shown evidence of decline in malaria parasitaemia and these could further buttress the low prevalence of malaria parasitaemia observed in our study.[158, 160,168]

The Nigeria malaria indicator survey (MIS) conducted in 2010[7] reported a much higher prevalence of 42% among under five children. In terms of age specific prevalence MIS reported 29.3% among 6-11 months old, 35.9% in 12-17 months, while the highest was among older children 48-59% months with a prevalence of 49%. In our study the overall prevalence among children less than 5 was 19% which is much lower than that obtained during the malaria indicator survey. This difference could be attributed to the fact that our study was health facility based while MIS was a community based survey with a much larger sample size. In our study HIV negative children aged 12-23 months old had a

prevalence of 26.2%. This was lower than that obtained from the MIS. The highest age specific prevalence found in our study was among children living with HIV aged 48-59 months with a prevalence of 42.7%. This corroborate with the MIS which found a prevalence of 49% among children aged 48-59 months. Children at this age are more likely to be found outside at evenings playing with pairs and expose to mosquito bite especially close to stagnant water and are also more likely not to sleep under an insecticide treated net even when it is available.[157]

Present findings of 23% prevalence among under fives, who are HIV negative is in contrast to the report of Gup *et al*[89] who found a prevalence of 80.3% among under fives in Eastern Nigeria and findings from Nwaorgu *et al* [88] who reported malaria parasitaemia prevalence of 76.4% among 1000 children in Awka, Nigeria. These two studies were conducted during the rains which is high transmission season. The sampling method in the two studies which involved children already with fever or malaria-related illnesses could account for these high figures recorded and may not be the exact reflection of the situation in the general population. Their findings nevertheless still attest to the fact that malaria is still a major health issue in this part of Africa.[88, 89]

The most predominant species found in this study was *Plasmodium falciparum* (95%). This findings confirms previous report that *Plasmodium falciparum* is the most prevalent species in Nigeria and accounts for about 98% of malaria cases in the country. [70] Reports from Tanzania indicates that *Plasmodium falciparum* was the predominant species found among febrile under five children.[84] Studies from Rwanda also reported *Plasmodium falciparum* as the species detected in one out of every six children of the 749 under fives surveyed.[92] These reports corroborates our findings in this present study.

From our study co infection of *plasmodium falciparum* and *malariae* was observed, also *Plasmodium ovale* among HIV positive children (4.8%). In our study the proportion of children under the age of five with malaria parasitaemia were asymptomatic. Similar studies have been reported from other malaria endemic areas[23, 158, 159] The presence of malaria parasites without febrile illness could be explained in part by the acquired protective immunity against malaria. In endemic areas protective immunity against malaria is acquired with an increase in exposure to malaria parasite which usually increases with age. [159]

Several factors have been described to predict malaria in children under the age of five in malaria endemic countries. Among these predictors are fever, duration of fever, intermittent fever and malaria parasitaemia.[129,160] Important environmental factors significantly associated with parasitaemia in this study was the presence of grasses surrounding the vicinity of the house where the child resides. This was significantly associated with malaria parasitaemia. Also the presence of pools of stagnant water close to the house where they child resides was statistically significant. This association can be explained partly that the presence of grasses (wet) and stagnant water provides a good breeding site for mosquitoes. This finding corroborates with reports of low malaria parasitaemia prevalence among children whose parent live in a clean and hygienic environment compared with children whose parents live in a hygiene compromised environment.[91]

The rate of usage of insecticide treated mosquito net among children living with HIV was 76.1% while usage rate among HIV negative children was 73.8%. This finding is much higher than that of the nationwide survey on the use of ITNs, which recorded 11.5%.[102] It

is also higher than the rate of usage in a study conducted in Makurdi among under five children that recorded net usage rate of 25%.[101] This difference in the rate of usage of insecticide treated net among under five children can be attributed to the fact that these studies were community based surveys covering a larger population. However our findings are similar to rate of usage of 62.4% reported among under five children in Gursum district in Ethiopia.[162] The usage of insecticide treated mosquito net among under fives in our study was good. This is probably due to massive radio and television campaigns on malaria prevention strategies and appropriate treatment options in Jos. Also health education talks during ART clinics and at the general outpatient department before commencement of each day's work.

Children who use insecticide treated mosquito net were less likely to have positive malaria slides. The protective effect of insecticide treated mosquito net use shown in this study adds to the vast body of evidence supporting the effectiveness of insecticide treated mosquito nets for protection against malaria and other vector-borne diseases.[105]

From our study it was observed that 92% of the children living with HIV who were on antiretroviral therapy were also taking cotrimixazole prophylaxis .The active ingredients in this drug (trimothoprim-sulfame-thoxazole) have been reported to be therapeutically effective against malaria parasite and therefore potentially protects against malaria infection.[163,164] This findings agrees with earlier reports by Longdoh *et al* from Cameroun who reported that 80% of his study participants who are living with HIV were on cotrimozaxole.[158]

The use of malaria rapid diagnostic test (M-RDT) is widely advocated for in Nigeria.[165] In our study only 6/42 (14%) of the positive malaria parasitaemia slides among children living with HIV was detected by the histidine rich protein 2 antigen for *Plasmodium falciparum*. Similarly among HIV negative under five children, 5/61(8.2%) of the *Plasmodium falciparum* was detected by RDT. Several studies have shown that malaria rapid diagnostic tests method have malaria parasites detection limit of 397-500 parasite/µl of blood against the 5 parasite/µl of blood detectable with Giemsa stained slide microscopically examined by competent and experienced microscopist.[166, 167]

Adequate knowledge of malaria symptoms and treatment among caregivers of children less than 5 years of age is essential in the management of malaria infection in these children.[132] In our study 95% of caregivers of children living with HIV had good knowledge of fever, malaria infection and management, while 97.7% of caregivers of HIV negative children had good knowledge. This finding contrast with previous reports of low level of knowledge of malaria infection among caregivers of under five children. [125, 128] Its however corroborates other studies that reported adequate knowledge of malaria and fever management among caregivers of under five children.[7,127, 129, 133] No association was found between CD4% and presence malaria parasitaemia among children living with HIV. This finding corroborates with report from Malawi[81] where no association was found between CD4 counts and clinical malaria among HIV positive individuals.

CHAPTER SIX – CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

In this study a low prevalence of 16% of malaria parasitaemia among children living with HIV was found. While among under five children who are HIV negative a prevalence of 23.11% was recorded. *Plasmodium falciparum* remains the most prevalent species responsible for cases of malaria infection among under five children from this study.

Important factors associated with malaria parasitaemia were the presence of grasses and pools of stagnant water in the vicinity of the house where the child resides.

The knowledge of fever, malaria infection and management among caregivers of under five children living with HIV and those without HIV was good.

The presence of anaemia was significantly associated with malaria parasitaemia among HIV negative under five children.

6.2 Recommendations

Government

1. With the satisfactory usage of insecticide treated mosquito net among under five children living with HIV and those who are HIV negative and the good knowledge of under five caregivers on fever, malaria infection and management recorded in this study, it is recommended that the government should intensify effort on the current massive radio and television campaigns through the roll back malaria initiative on malaria prevention strategies in Jos. This can further influence the reduction of malaria parasitaemia prevalence among these children.

2. Caregivers of under five children

   Parents of children less than five years should intensify efforts in ensuring clean environment devoid of stagnant water and grasses as this will reduce the breeding site for mosquitoes

   Health care providers

3. With the low malaria parasitaemia prevalence and low transmission observed from this study, we recommend that the practice of treating children less than five years presenting with febrile illness with antimalarial should be done only when the diagnosis is confirmed by microscopy by the health care providers in the respective health facilities. This will further reduce the number of malaria parasitaemia negative children given antimalarials.
4. Where possible the use of Rapid Diagnostic Test for the diagnosis of malaria by clinicians in the health facilities should be confirmed with microscopy as not all RDT negative test are without malaria parasitaemia.

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APPENDICES

Appendix 1: INFORMED CONSENT FORM

Consent to Participate in Malaria Infection Study

Olawuyi Kayode, a resident of Nigerian Field Epidemiology and Laboratory Training Program (Federal Ministry of Health) in conjunction with Department of Community Medicine, Faculty of Medicine Ahmadu, Bello University Zaria, is conducting a study to determine the prevalence of malaria infection among children who are less than five years of age. You are invited to present your child to participate in this study and your participation is completely voluntary. Your decision to either take part or not in the study will NOT affect the type of service that you receive at this clinic.

What is the Purpose of this Study?

Malaria infection is a serious and sometimes fatal disease that is caused by a parasite called Plasmodium. These parasites are carried by mosquitoes who became infected from biting someone who already has the disease. Malaria is transmitted to other people when the infected mosquitoes bite them. Children who are infected with malaria become very sick with fever, shaking chills, tiredness, and sometimes vomiting and diarrhea may occur. Malaria is curable. The purpose of this study is therefore to detect the presence of malaria parasite in the blood of your child, so as to treat immediately before any form of complication set in.

Who will take part in the study and for how long?

Children who are 6 months old and above but not more than the five years of age diagnosed to either be receiving treatment in this clinic.

What will be done to me?

Should you accept to participate in this study, as part of the routine check up on your child's health, 3mls of blood will be collected from your child to test for the presence or absence of this parasite using a standard testing instrument known as Microscope. Other test such as haemoglobin test and CD4 % will be done as well. To identify which type of the parasite is found in your child's blood in order to know which drug will be most appropriate, a blood smear will be made on a clean slide and examined using the microscope. The body temperature of your child will be taken to establish the presence or absence of fever. Other tests and treatment your child receives from this clinic will continue whether you decide to allow your child participates or Not. Also you will be asked some further questions about your child and general social life at home as it relates to malaria prevention and control.

Confidentiality

Any information that is obtained in connection with this study and that can be identified with your child will be kept secret and will be disclosed only with your permission .Confidentiality will be maintained by means of a code number. We will not use your child's name in any of the information we get from this study or in any of the research reports.

What are the Benefits and Risks?

All parents/caregivers will be educated on the current National guideline for the prevention of malaria infection especially among under- fives. The diagnosis of malaria infection and appropriate referral for treatment and management at no cost to the participants. The result of the test will also provide useful information for Nigeria

National Malaria control program on the burden of malaria parasite among children less than five years of age. Your child may experience a little discomfort during the blood collection process.

Participation and Withdrawal

You can choose whether or not to be in this study. If you volunteer to be in this study, you may withdraw at any time without consequences of any kind. You may also refuse to answer any questions you do not want to answer. There is no penalty if you withdraw from the study and you will not lose any benefits to which your child is otherwise entitled.

Identification of Investigator

You can ask any questions that you have about the study. If you have a question later that you didn't think of now, you can call Kayode Olawuyi on 07038337570 or 08053020011.Email:- abkay2008@yahoo.com

I understand the concept of the research described above. My questions have been answered to my satisfaction, and I agree to allow my child participate in this study. I have been given a copy of this form.

_____ Date _____

Printed Name and signature of caregiver
(Authorize representative)

_____ Date_____

Name and Signature of Investigator

_____ Date _____

Witness

Appendix 2: QUESTIONNAIRE

COMPARISON OF MALARIA PARASITAEMIA AMONG CHILDREN LESS THAN FIVE YEARS LIVING WITH HIV AND THOSE WITHOUT HIV IN HEALTH FACILITIES IN JOS, NIGERIA

Date..... **H/FACILITY:**

QUESTIONNAIRE NO[ ]

INTRODUCTION

Good day. Olawuyi Kayode, a resident of Nigeria Field Epidemiology and Laboratory Training Program (Federal Ministry of Health) in conjunction with Department of Community Medicine, Faculty of Medicine Ahmadu Bello University Zaria, is conducting a study to determine the prevalence of malaria infection among children who are less than five years of age. This is to access factors that contribute to malaria infection and how to control it. You are invited to respond to the questions below with respect to your child. This interview will take about 12 minutes. Please note that participation is voluntary and you are at liberty not to answer any question you do not feel like answering. Information you provide will be kept confidential. Thank you.

A: **SOCIO-DEMOGRAPHIC DATA:**

1. Respondents relationship with child……
2. Age of respondent at last birthday (in years)……
3. Gender [ ] Male [ ] Female

4. Marital status [ ] Single [ ] Married [ ] Widowed [ ] Separated [ ] others (please specify)……

5. Level of education [ ] No formal education [ ] Primary school [ ] Secondary School [ ] Tertiary (please specify ND, HND, B.Sc, M.A etc.)……

6. Occupation [ ] Employed for wages [ ] Self employed [ ] Unskilled labor (petty trading) [ ] unemployed [ ] housewives [ ] others (please specify)……

7. What is your present personal income level per month? Below N18, 000 [ ] N18, 000-N36, 000 [ ] N36, 000-N 54,000 [ ] N54, 000-N120, 000 [ ] Above N120, 000.

8. Number of children in the family.....

9. Position of child in family.....

B: CHILD HEALTH-RELATED INFORMATION

10. Age of child (in months)……

11. Sex of the child [ ] Male [ ] Female

If child is HIV negative, skip and go to question 16

12. Weight (Kg) [ ] Height (M) [ ] MUAC Taken [ ] T^0 [ ]

13. When was this child diagnosed of HIV infection ……

14. When did he/she commence the antiretroviral therapy? ……

15. Is/he taking cotrimoxazole? Yes [ ] No [ ] If yes for how long? Please specify……..

C: KNOWLEDGE ON MALARIA AND FEVER MANAGEMENT

16. What do you think causes fever in Children? Circle as appropriate (a) drinking dirty water (b) change in weather (c) teething (d) It is natural for children to have fever (e) Bacterial infections. Others specify…….
17. How do children get malaria infection? Circle as appropriate (a) when bitten by infected mosquito (b) when exposed to cold weather (c) exposure to stagnant water (d) playing with other children (e) others specify……
18. What are some things that can happen to a child when he/she has malaria? Circle as appropriate: (a) Fever (b) Rigor and chills (c) Rashes (d) Muscle aches (e) (f) Flu-like illness (g) Poor appetite ……
19. When your child present with fever (increase in body temperature above 37^0C) typically what is your first response? Circle as appropriate:

(a) Home management with local herbs (b) Visit patent medicine store (c) Go to the nearest Clinic/hospital (d) Give any available drug (what drug?)…… (e) Tepid sponge, administer antipyretics and go to clinic/hospital (f) Others specify …….

20. Typically how long does it take from when your child present with fever and when you seek help from an approved health facility? Circle as appropriate Immediately (b) 2- 3 days (c) 3-4 days (d) I do not take the child to hospital

21. When you want to buy drug for a child with malaria fever, which of these is most important? (Only one response)

[ ] Paracetamol [ ] Antimalarial (CQ, SP, ACT, etc)

[ ] Haematinics /multivitamins [ ] Antibiotic [ ] others

22. Have your child taken any anti malaria drug in the past two weeks?

Yes [ ] No [ ]

If yes which drug? ……..

D: RISK FACTOR FOR MALARIA INFECTION IN CHILDREN

23. How many are you in your household? ……

24. Are there window and door nettings in your house where this child sleeps?

Yes [ ] No [ ]

25. Does your household have insecticide treated net mosquito net that can be use while sleeping? Yes [ ] No [ ]

26. If yes how many nets do you have in your household? Circle as appropriate

(a) 1 (b) 2 (c) 3 (d) more than 3 (e) I do not know

27. If no, why is your household not having mosquito nets? Circle as appropriate

(a) don't like to use nets (b) it is not readily available (c) Mosquito net is too expensive (d) I do not need mosquito net (e) others specify……

28. When was the last time your child slept under an insecticide treated mosquito net? Circle as appropriate: (a) Yesterday (b) one week ago (c) two weeks ago (d) One month ago (e) more than one month ago (f) Never use net

29. Around your household, are there pools of stagnant water, stream/river, grasses and refuse dump sites?

    (a) Yes [ ] (b) No [ ]

30. In the past two months how many episodes of malaria infection has your child suffered? Circle as appropriate (a) One (2) two (c) three (d) four (e) None. If none go q27

31. How many times during these episodes of malaria did your child have a drop of blood taken from his/her fingers for testing in the laboratory? Circle as appropriate (a) none (b) once (c) twice (d) more than two times.

32. Has the child been hospitalized before on account of malaria fever?

    Yes [ ] No [ ]

33. If yes on the average, how much did you spend for treatment in naira? (a) <1000 (b)1000-2000 (b) 2000-5,000 (c) above 5,000

34. If no to Q28 above, on the average how much is the out of pocket expenses you incur in naira when ever this child has malaria fever? (a) < 500 (b) 500-1,000 (c) 1,000-2,000 (d) 2,000-5,000 (e) above 5,000

35. Has the child travelled out of this LGA in the past 21 days? Yes [ ] No [ ]

If yes, please name place visited……

∞∞∞∞ ----------

Child's name/APIN No …….

Blood sample collected Yes [ ] No [ ]

Questionnaire number written on Blood sample [ ]

APPENDIX 3: ETHICAL APPROVALS

JOS UNIVERSITY TEACHING HOSPITAL
JOS, NIGERIA

Phone: 073-450226 - 9
E-mail:juth@infoweb.abs.net

Cables & Telegram: JUTH
P.M.B. 2076
Jos.

Ref: JUTH/DCS/ADM/127/XXII/ 5457

Date: 15th January, 2013.

Olawuyi Abraham Kayode,
Department of Community Medicine,
Faculty of Medicine,
Ahmadu BelloUniversity Zaria,
Kaduna-Nigeria.

RE: ETHICAL CLEARANCE/APPROVAL

I am directed to refer to your application dated 19th December, 2012 on the research proposal titled:

"Prevalence of Malaria Infection among HIV Positive under 5 Children Attending ART Clinic at PEPFAR Sites in Jos Metropolis, Plateau State " and your appearance before the Ethical Committee on 28th December, 2012.

Following recommendation from the Institutional Health Research Ethical Committee, I am to inform you that Management has given approval for you to proceed on your research topic as indicated.

You are however required to obtain a separate approval for use of patients and facilities from the department(s) you intend to use for your research.

The Principal Investigator is required to send a progress report to the Ethical Committee at the expiration of three (3) months after ethical clearance to enable the Committee carry out its oversight function.

Submission of final research work should be made to the Institutional Health Research Ethical Committee through the **Secretary in Room 14, Administration Department,** please.

On behalf of the Management of this Hospital, I wish you a successful research outing.

Hajia R. Danfillo
For: *Chairman, MAC*

INSTITUTIONAL HEALTH RESEARCH
ETHICAL COMMITTEE
DESPATCHED
15 JAN 2013
JOS UNIVERSITY TEACHING
HOSPITAL P.M.B 2076 JOS

Faith Alive Foundation

(Non-Profit, Non-Governmental Medical & Social Ministry)

All Correspondence to **THE FOUNDING CO-ORDINATOR**

*Our Ref*_____

*Your Ref:*_____ *Date:*_____

OUR REF FAF/ECC/22-11

5th September, 2013

Olawuyi Abraham Kayode

Department of Community Medicine

Faculty of Medicine

Ahmadu Bello University Zaria

Kaduna-Nigeria

ETHICAL CLEARANCE/APPROVAL

Following your application and appearance before the ethical committee, I write to convey the approval of the committee after consultations with the Health Unit of Jos North Local Government of Plateau State of your Research Proposal titled **"Prevalence of Malaria Infection among Children less than 5years living with HIV in selected Health facilities in Jos"**

Please adhere to the stated protocols as mutually agreed and inform the committee of the progress of your work after every six months including submission of the final research work. This will enable the committee to carry out its oversight function.

This ethical clearance is valid for six months after which you will have to re-apply for extension if required.

On behalf of the Committee and Management of Faith Alive Foundation, I wish you a successful research adventure.

Yours Faithfully,

SIGN 5/9/13

DATE

Segun Adeyanju

Secretary, Faith Alive Ethical Committee

Come over...and help us. Acts 16:9

Faith Alive Social Services & Chaplaincy
3B Eyamba Street,
Jos Plateau State, Nigeria.

Faith Alive Hospital & PMTCT Center,
29 Zik Avenue, P. O. Box 745 Jos, 930001
Plateau State, Nigeria
Tel: 234-73-452393, 451602, 460319
E-mail: cogom@hisen.org

Faith Alive Outreach Centers
- Kafanchan
- Bakin - Kogi &
- Yola.

PLATEAU STATE SPECIALIST HOSPITAL JOS

Ref. No. PSSH/ADM/ETH. CO/2013/109
Old Bukuru Road,
P.M.B. 2113,
Jos, Nigeria
Tel. 073-462180
Fax: 073-464031

Email: psshjos@yahoo.com

Date: October 23, 2013

Reg.No: NHREC/05/01/2010b
Notice of Expedited Review and Approval

Re: Malaria infection among children less than five years living with HIV attending ART clinic at selected health facilities in Jos, Nigeria.

Name of Principal Investigator: Olawuyi Abraham Kayode

Address of Principal Investigator: Dept. of Community Medicine, A.B.U., Zaria, Kaduna State

Date of receipt of valid application: 09 September, 2013

Date of meeting when final determination of research was made: 23 October, 2013

This is to inform you that the research described in the submitted protocol, has been reviewed and *given expedited approval by the Health Research Ethics Committee.*

This approval dates from **23/10/2013 to 22/10/2014**. Note that no participant accrual or activity related to this research may be conducted outside of these dates.

You may liaise with the ART clinic Project Coordinator.

The National Code for Health Research Ethics requires you to comply with all institutional guidelines, rules and regulations and with the tenets of the Code including ensuring that all adverse events are reported promptly to the HREC. No changes are permitted in the research without prior approval by the HREC except in circumstances outlined in the Code. The HREC reserves the right to conduct compliance visit to your research site without previous notification.

Dr. Bitrus Matawal, MBBS, FWACS
Chairman, HREC PSSH

APPENDIX 4: WORK PLAN

Work Plan for studies on "Comparison of Malaria Parasitaemia among Under five Children living with HIV and those without HIV in Selected Health Facilities in Jos, Nigeria"

Activity	Resources	Persons responsible	Indicator	Means of Verification	Source of funding	Time frame
Writing and approval of research proposal		Principal investigator	Approved research proposal	Research proposal printed	NFELTP	April-June 2013
Obtain ethical clearance						July-August, 2013
Consultation and orientation of selected health facilities	Transport, Laptop, health facility	Principal investigator	Principal officers in health facilities oriented	Number of staff oriented		September, 2013
Purchase of reagents/materials	Quotations, invoices and funds	Principal investigator	No of reagents and consumables purchased	Reagents and material available		September, 2013
Training of research assistants	Study protocol, LCD, projector, laptop	Principal investigator	No of research assistant trained	Attendance list		September,2103
Collection of data	Under 5 children and care givers, questionnaires	Principal investigator, research assistants	No of films made	Number of consent forms signed		October, 2103-Febuary, 2014
Lab investigation	Lab, PPE, microscope, counting chamber,	Principal investigator and research assistants	Stained and viewed slides	All results entered into the report forms		October, 2103-Febuary, 2014
Enter and clean data	Laptop, filled questionnaire lab results	Principal investigator	No of questionnaire entered into Epi info database	All data correctly entered		March- April, 2014
Analysis of data	Laptop, filled questionnaire	Principal investigator	No of variable analyzed	Report generated		March-April, 2014
Write report		Principal investigator	Thesis submitted	Thesis submitted on time		May 2014