ASSESSMENT OF THE ROLE OF MALARIA IN THE AETIOLOGY OF RENAL IMPAIRMENT IN ISU COMMUNITY IN EBONYI STATE, NIGERIA

BEING A POSTGRADUATE RESEARCH THESIS SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF A DOCTOR OF PHILOSOPHY (PH.D) DEGREE IN PARASITOLOGY OF THE DEPARTMENT OF ZOOLOGY AND ENVIRONMENTAL BIOLOGY, UNIVERSITY OF NIGERIA NSUKKA

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CERTIFICATION PAGE

Ani, Ogonna Christiana, a postgraduate Ph.D student in the Department of Zoology and Environmental Biology, with Registration Number PG/Ph.D/05/40230 has satisfactorily completed the requirements for the award of the degree of Ph.D in Parasitology. The contents of the thesis is original and has not been submitted in part or full for other certificate, diploma or degree of this or any other university elsewhere.

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DEDICATION

I dedicate this work to God Almighty, the owner of my life.

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ABSTRACT

This study, the involvement of malaria in renal impairment among individuals in selected villages of Isu community in Onicha Local Government Area of Ebonyi State was investigated. A two-stage sampling design was adopted in which selection of villages constituted the first stage/Primary Sampling Units(PSUs) where three (3) villages (Isuachara, Agbabor and Mgbala-ukwu) out of the seven villages were selected using simple random sampling. In the second stage, a simple random sample of 240 individuals was taken from the three villages selected, using 95% confidence level and a margin of error of 6.32% with a standard deviation of 0.5 .Thick blood smears of venous blood stained with Giemsa were examined microscopically for malaria parasitaemia (MP) and its intensity. Malaria negative individuals served as controls. Biochemical parameters (Total protein, serum creatinine, urea and albumin), haematological indices (packed cell volume (PCV)), total leucocyte counts (TLC) and differentials and electrolytes of malaria positive and negative individuals were determined using standard procedures. Ultrasonographic examination of kidneys was carried out to compare the features and sizes of both malaria positive and uninfected individuals. Malaria positive individuals were treated with current anti-malaria drugs and post treatment examination of the parameters were carried out three weeks after the treatment. This is to investigate the effects of treatment. The overall prevalence and parasite intensity in the study population were 37.5% (n = 90) and 2361$\pm$857.55p/µL respectively. most of the infected individuals had mild infection. There was no difference in infection rate by sex. Among the age groups, the prevalence of malaria parasite was higher in younger individuals but the differences were not statistically significant (P>0.05). Biochemical parameters of malaria positive individuals were higher than those of the uninfected. The differences were statistically significant (P<0.05) except for the albumin level (P> 0.05). Packed cell volume and monocytes of malaria infected individuals were higher and differed significantly from those of uninfected individuals (p<0.05). There was no significant difference in the concentration of electrolytes, SG and pH of malaria positive and negative individuals (P>0.05). The electrolytes, SG and pH of malaria positive and negative individuals did not differ statistically (p>0.05). Among the age groups, there was no marked difference in the biochemical, haematological and electrolyte levels of malaria positive and negative individuals (P>0.05). In malaria positive individuals, correlation analysis showed that there was positive but insignificant association between the intensity of malaria parasitaemia and total protein and albumin (r=0.0.198 and r=0.052) respectively while statistically significant positive association existed between malaria intensity, urea and creatinine (r=0.348 and r=0.602). The total leucocyte count, packed cell volume and eosinophil count also showed mild significant association with intensity of malaria parasite while no significant association existed between intensity of malaria parasite and electrolytes. After treatment, the prevalence of malaria parasite was drastically lowered to 9.1% with those still infected having only mild infection (26.3%). Biochemical parameters were also lowered and significant differences were observed in all of them (P< 0.01) while there was no significant difference between malaria parasite and the electrolytes, the SG and pH levels (P>0.05). The mean values of haematological parameters significantly reduced except the packed cell volume. Only the TLC count and eosinophils, showed significant association with MP while electrolytes did not. The ultrasonographic imaging of the kidneys of malaria infected and uninfected individuals showed that malaria parasitaemia altered the sizes of the kidneys of malaria positive individuals and some of them lost their cortico-medullary differentiation. Malaria

infection may therefore be said to contribute to renal impairment as treatment of infected individuals with anti-malaria drugs significantly lowered the parameters that may lead to renal problems. It is therefore recommended that the diagnosis of malaria and renal function should go hand in hand in our health institutions. Renal impairment due to malaria infection will thus be prevented and recovery of diagnosed patients may be achieved by early treatment of malaria. This will invariably lead to reduction in both mortality and morbidity rates of both malaria infection and renal impairment.

CHAPTER ONE
INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

1.1.1 Background History of Malaria

The term malaria originated from Italian: meaning bad air and the disease was called ague or mesh fever due to its association with swamps in humid regions of the world (Cheesbrough, 1999). It is an ancient disease that has plagued humans throughout history (Joy *et al.*, 2003). It is a term used for acute or chronic infection caused in man or other vertebrates such as mice and donkeys. Malaria remains one of the most pressing health problems in the world with an estimated 300-500 million cases annually of which 90% occurs in Africa (Tarimo *et al.*,1998). It is the most important infection of great global public health concern and by far the world's most important tropical parasitic disease that kills more people than any other communicable disease except tuberculosis(Yah *et al.*, 2005). According to WHO (2000) about 500 million people are affected by malaria at any time and approximately 2 million of them mostly children die each year (Raven and Johnson, 2002; Onyesome and Onyemakonor, 2011).

The global malaria situation continues to show no real improvement. Downward trends in the number of reported cases are maintained in some countries but are counter balanced by increasing trends in others. According to WHO (1987), there has been very little change in the geographical distribution of malarious areas but within countries, areas of rural economic exploitation have become foci for intense malaria transmission. The malaria situation in sub-Saharan Africa is grim and the disease constitutes a leading cause of poverty in the region (Wenceslaus, 2000). This is because African region has the greatest number of people exposed to stable malaria transmission and the greatest burden of malaria morbidity and mortality in the world (WHO, 1996; Snow *et al.*, 1999). The problems associated with malaria

treatment in Africa and drug resistance also increase the rates of severe illness and death substantially (Peter *et al*., 2000).

Although accurate figures are difficult to come by, it is estimated that in Africa alone malaria is responsible for one million death of infants and young children each year (Angyo *et al.*, 1996). With regards to morbidity, people in areas of high endemicity usually go through several attacks every year. Each attack may last about 5 to 15 days often incapacitating the victim. In highly endemic areas, most cases of severe malaria occur among children aged six months and five years with the highest mortality in those between one and three years of age. Another risk group in endemic areas are pregnant women who become susceptible to severe infection due to diminished cellular and humoral immunity during pregnancy (Anorlu *et al*.,2001; Okwa, 2003; Adefioye *et al*., 2007; Uneke, 2008). This is because pregnancy is usually accompanied by physiological and immunological changes that modify both resistance to infection and the pathogenesis of the disease.

The four species of the parasite that infect man are *Plasmodium falciparum, P. vivax, P. malariae* and *P. ovale. Plasmodium falciparum* and *P. vivax* are the most common in the tropics but mixed infection with two or more of the *Plasmodium* species is common. Blood stage cycle of *Plasmodium falciparum* is responsible for most cases of malaria and for the most severe, often fatal forms of the disease. It has varied modes of presentation with occasional life threatening complications. It is said to be complicated when any one or more of the clinical features such as cerebral malaria, jaundice, renal failure, pulmonary oedema, hypoglycemia, circulatory collapse, spontaneous bleeding, repeated generalized convulsion and acidosis are manifested (Bag *et al.*,1994). Severe falciparum malaria remains an important cause of mortality in the tropical world with an annual mortality of 1-2.7 million people and a mortality rate as high as 15-30%, despite effective anti-malarial

treatment (WHO, 1990; 1993 and 2013). It is also found to be deeply entrenched in tropical Africa and highly endemic in sub-Saharan Africa.

Plasmodium vivax is the commonest species in South America and Asia while *P. malariae* and *P. ovale* typically cause a relatively benign infections and rarely occur in these areas. Malaria infection may be acquired wherever there are human hosts carrying the parasites and a sufficiency of suitable *Anopheles* mosquitoes together with conditions of temperature and humidity which favour the development of the parasite in the mosquitoes. *Plasmodium* has strategies for interacting with human immune system in the following ways.

i. Antigenic variation in malaria parasite

ii. A defined splenic dependent regulation of parasite genes in coding structural proteins and adhesive molecules on the erythrocyte surfaces that are involved in their adherence to the endothelium.

iii. Low immunogenicity of conserved parasite peptides that are targets of antibodies able to interfere with parasite survival.

Malaria affects almost all organ systems (e.g lungs, liver, spleen, brain and kidney). Clinically, significant renal involvement is associated with infections by *Plasmodium falciparum* and *Plasmodium malariae.* Cases of malaria associated renal impairment have been reported from different parts of the malaria endemic countries (Mishra *et al.*;2003, Sharma *et al.*; 2004; Ogbadoyi and Gabi, 2007). Such impairments most of the time lead to nephrotic syndrome or nephrosis. Nephrotic syndrome is a group of symptoms caused by the excretion of large amount of protein in the urine (>3g of protein /day) due to kidney impairment or glomerula disorder. It occurs at any age and is characterized by such symptoms as high levels of protein and high levels of cholesterol and lipids in the blood, retention of fluid in the tissues (oedema) and appearance of fat in the urine. Its causes may include the disease that cause glomerulonephritis (kidney inflammation), systemic diseases such as diabetes or

disease of unknown origin which causes a form of the syndrome known as idiopathic nephrotic syndrome. Nephrotic syndrome progresses to kidney failure over several years. Such chronic infection can also be associated with an increased incidence of vascular disease due to the high fat levels in the blood. The most common renal lesion of malaria is acute renal failure due to acute tubular necrosis. This is seen in about1% of all *P. falciparum* infected patients and its incidence rises to as high as 60% in patients having heavy parasitaemia (Rajapurka, 1994). The severity of malaria-induced renal impairment in a particular area is largely a function of the disease prevalence and other aetiological factors prevailing in the area. Infection with *P. falciparum* produces only acute manifestations which range from asymptomatic urinary abnormalities and mild electrolyte disturbances. The contribution of falciparum malaria to the total admission with acute renal failure depends to a large extent on the prevalence of disease, referral pattern and other aetiological factors present in the area under study (Barsoum, 2000).

The two major renal syndromes associated with malaria are chronic and progressive glomerulopathy that are usually associated with complicated quartian malaria associated with falciparum malaria. Chronic malarial nephropathy affects children usually 4 to 8 years old and presents as a steroid- resistant nephritic syndrome. Its characteristic histopathologic lesion is mesangio-cappillary glumerulonephritis with subendothelial immune complex deposites containing IgG, C3 and malarial antigens. This proceeds to renal failure even after successful eradication of the infection. It is much more frequent in non-immune population where 25 to 30% cases are reported.

In tropical developing countries, almost the entire population is deemed to be under malaria risk. It is emerging as an important problem in tropical countries and carries a high mortality, especially when the disease is not diagnosed early, referral to

health centre having dialysis facility is late or when dialysis facility is not available in the hospitals or health centres.

In Nigeria, malaria accounts for 30 – 50% of out patients in health institutions across the country and 8-10% admitted children are due to malaria with mortality rate of 0.3 million annually (Matur, *et al*., 2001; WHO, 2008; Ogbadoyi and Tsado, 2009; Onyesome *et al*., 2010).

1.1.2 JUSTIFICATION OF THE STUDY.

Information on the association of malaria with impairment of renal function in Nigeria is scanty but very necessary because malaria is highly endemic in the country. The mortality and morbidity rates of the infection are also high especially in high risk groups such as children and pregnant women. Unfortunately , this information is very scanty in Nigeria and has not been investigated in Ebonyi State. Renal failure (Serum creatinine >3mg/dl) and jaundice (bilirubin>3mg/dl) may be indicative of severe malaria and therefore requires special attention. In Nigeria, cases of inadequate treatment of malaria is quite high among the urban and rural poor. This is because of the high level of self medication that arise from difficulties inherent in weak health care system which make people to report to hospitals as a last resort. Majority of Nigerians and the urban and rural poor in many countries of sub-Saharan Africa also use local herbs and plants as their main sources of medicines instead of complementary sources. Though some of these herbs may be very effective, others are not and the implication is that the impairment of organ functions which would have been transient, present only during the duration of the disease, will gradually progress to chronic organ dysfunction with its disastrous consequences .It therefore becomes absolutely necessary that malaria involvement in renal impairment in Nigeria be assessed. This will ensure proper management of malaria infection with its associated complications.

1.1.3 Objectives of the Study

Against the background of the information stated above, this study is aimed at the following objectives.

Main objective

To determine the effects of malaria infection on renal function among malaria patients in Isu Community, Onicha Local Government Area, Ebonyi State.

Specific Objectives

1. To measure the overall prevalence and intensity of renal impairment in malaria and non malaria patients by age and sex.

2. To investigate changes in health determinants among malaria infected and non malaria infected individuals in correlation with intensity of malaria infection.

3. To determine the relationship between malaria parasitaemia and kidney features and functions in malaria patients.

1.2 LITERATURE REVIEW

1.2.1 Epidemiology and Incidence of Malaria

The geographical area affected by malaria has shrunk considerably over the past 50 years but control is becoming more difficult and gains are being eroded. According to WHO (1999), increased risk of the disease is linked with changes in land use linked to activities such as road construction, mining, logging and agricultural and irrigation projects particularly in “frontier” areas such as the Amazon and South-east Asia. Other causes of its spread include global climatic change, disintegration of health services, armed conflicts and mass movements of refugees. The emergence of multi-drug resistant strains of the parasite is also exacerbating the situation. Through international travel, imported cases of malaria are now more frequently registered in developed countries hence malaria is re-emerging in areas where it was previously under control or eradicated (Salako,1991).

The current global picture of malaria shows that:

i. Malaria is a public health problem today in more than 90 countries inhabited by a total of some 2,400 million people (that is 40% of the world’s population).
ii. World wide prevalence of the disease is estimated to be in the order of 300-500 million clinical cases each year.
iii. More than 90% of all malaria cases are in sub-Saharan Africa (Hay *et al*., 2004).
iv. Mortality due to malaria is estimated to be over 1million deaths each year. The vast majority of deaths occur among young children and pregnant women in Africa, especially in remote rural areas with poor access to health services.
v. Other high-risk groups are non-immune travelers, refuges, displaced persons and labourers entering endemic areas.

vi. Malaria epidemics related to political upheavals, economic difficulties and environmental problems also contribute in the most dramatic way to death tolls and human suffering.

vii. Malaria is endemic in a total of 101 countries and territories: 45 countries in WHO's African Region, 21 in WHO's American Region, 4 in WHO's European Region, 14 in WHO's Eastern Mediterranean Region, 8 in WHO's South-East Asia Region and 9 in WHO's Western Pacific Region (WHO,1998).

The prevalence of malaria in an area is governed by the density of mosquito population, presence of people especially children having gametocytes within their red blood cells, presence of environments that support mosquito breeding, effectiveness of anti mosquito measures and the use of suppressive drugs (WHO, 1985). Other factors that affect the prevalence of malaria in an area are parasite species and strains, human population dynamics, economic conditions, climate and land use. Dry and desert areas are not favourable for malaria. Transmission of malaria therefore is highest during the rainy season (Imboua-Bogui and Diawara 1986; Eneanya,1998 and Uneke *et al*., 2006). The suitable climate, geographic and hydrographic conditions favourable for malaria transmission include temperature of 20^{o}C-28^{o}C, mean monthly rainfall greater than 10cm, relative humidity greater than 60% and a topography with altitude less than 200 meters above sea level. The development of larval stage of mosquitoes occurs in pools of stagnant water which is abundant during the rainy season. These factors also determine the levels of malaria endemicity and the development of immunity.

The success of *Plasmodium* as a human parasite is also in large part due to the slowly acquired and only partially protective nature of the host immune response. All individuals exposed to malaria parasite either by visiting or living in malaria endemic areas but are not immune to the disease are at risk. Even among those

who are not immune to malaria and are living in endemic areas, studies have shown that it is the under privileged in those areas who are more at risk (Munguti, 1998).

In the vast majority of tropical and sub-tropical regions of the world, malaria remains the most complex and overwhelming health problem facing humanity with 300 to 500 million cases and 2 to 3 million deaths annually (WHO, 2000) and forty percent of the world's population lives in these endemic areas. It remains a major global public health problem and a major cause of morbidity and mortality in many countries of the tropical world. It is the world's most important parasitic disease and ranks among the major health and developmental challenges facing large parts of the world, including some of the poorest countries.

Despite the fact that malaria is a preventable and curable disease and that treatment is currently cheap, it is still one of the leading causes of death in many developing countries (Meek *et al*., 1998) and there has been little change in which areas are at risk of the disease since 1992. Precise statistics are unknown because many cases occur in rural areas where people do not have access to hospital or the means to afford health care. As a result, the majority of cases are not documented. About 90% of all malaria deaths in the world today occur in Africa South of the Sahara (Hay *et al*., 2004). This is because the majority of infections in the region are caused by *Plasmodium falciparum,* the most dangerous of the four human malaria parasites and also because the most effective malaria vector-the mosquito-*Anopheles gambiae* is the most wide spread and the most difficult to control (WHO, 2003). Most people at risk of infection in sub-Saharan Africa live in areas where vector transmission of malaria is common and occurs with sufficient frequency than other potential routes of transmission (WHO, 2000).

In Africa, malaria is present in both rural and urban areas though the risk is lower in the larger cities (Keiser *et al*., 2004). It causes about 400-900 million cases of fever and approximately 1-3 million deaths annually (Breman, 2001). This represents at

least one death every 30 seconds. The vast majority occur in children under the age of 5 years and pregnant women. According to Greenwood (1999), a visit to the pediatric ward or to the general out-patient department of most hospitals in tropical Africa is all that is needed to convince a visitor that malaria remains a major cause of mortality and morbidity in African children. It precipitates terrible mutilating afflictions in children and leads to numerous complications such as anaemia, pulmonary oedema, renal failure (Eze and Mazeli, (2001). WHO (2000a) reported that the lives of some 500,000 African children might be saved each year if insecticide impregnated mosquito nets were widely and properly used. This lends credit to the same fact that malaria is a serious threat to life of African children. Most of the mortalities and morbidities are associated with complicated cerebral malaria, metabolic acidiosis and severe anaemia (Egri-Okwaje *et al*., 1999).

Cerebral malaria continues to be the most severe and life threatening complication of falciparum infection in children in holo-endemic areas (Sluster *et al*.,1994, Waller *et al*.,1997). In Dar es Salam, Okeahialam, (1972) reported high prevalence of malaria infection among children of school age while in Humera, North Western Ethiopia malaria is also a threat to the lives of children of school age (Seboxa and Snow 1997). They noted that of the 705 paediatric admissions to Humera District Hospital of children aged one month-ten years, 458 (68%) were clinically regarded as having a primary diagnosis of malaria. The case fatality rate among paediatric malaria admissions was also high as 99 of the children died while in the ward. In Benin Republic, Valema *et al*.,(1991) reported that the average number of fever episode per child per year was 2.4 and that 33% of this was estimated to be caused by malaria. Malaria is also one of the major health problems in Ghana accounting for 7-8% of all certified deaths and ranks 5th as the commonest cause of death in children less than 5 years (Amofa and Adjei-Acquua, 1996).

In areas where malaria is endemic, pregnancy is associated with increased susceptibility to malaria. Malaria during pregnancy poses a substantial risk to both the mother, her foetus and the neonate (Cot and Deboron, 2003).This risk ends with delivery and decreases with the number of pregnancies. Walker-Abbey *et al*., (2005) carried out a research on malaria among pregnant Cameroonian women and reported high infection rate and also noted that transmissions were estimated to be 13 infectious bites per year. Bouyou-Akotet *et al*., (2003) also investigated the prevalence of *Plasmodium falciparum* infection in pregnant women in Gabon and reported that both malaria and anaemia were high in this risk group. In such malaria endemic areas, the prevalence of clinical and asymptomatic malaria is highest in young women and those in their first and second pregnancies (McGregor, 1984 and Menendez, 1995). However, with successive pregnancies, women acquire a gravidity-dependent form of immunity resulting in a decrease in both prevalence and severity of infection.

Malaria is holo-endemic in all parts of Nigeria. WHO estimated that 103 countries in the world have an on-going malaria transmission and only 62 of these countries are at the point of eliminating it but unfortunately Nigeria is not one of them. It remains one of the major public health problems in the country and the burden is on the increase in spite of numerous interventions that have been instituted. The obstacles to the success of these interventions are socio-cultural, economic and political in nature. *Plasmodium falciparum* causes most severe malaria illness and death and is the commonest and most devastating in Nigeria. Two broad categories of predisposing factors are implicated in the prevalence of malaria in Nigeria. These are behavioural and non-behavioural factors. The behavioural factors relate to some cultural practices that promote mosquito breeding and access of mosquitoes to the people as well as failure of vulnerable populations to use proven and effective interventions for the treatment, control and prevention of malaria. The main non-behavioural factors include geographical or ecological peculiarities such as tropical

(warm) climate and vegetation that promote breeding and sustenance of mosquitoes and the causative agent.

Malaria is a major cause of morbidity and mortality in Nigeria. At last 50% of the population suffers from at least one episode of malaria each year and the disease is the commonest cause of out-patient attendance across all age groups (Salako *et al*.,1990). It accounts for up to 25% infant mortality and 30% childhood mortality (Afolabi *et al*., 1997). It was established from hospital records that 8-9% of hospital attendance in Nigeria is due to malaria infection.

School age children in all parts of the country have an asymptomatic parastite density. Adeyemo *et al*., (1999) recorded 80% asymptomatic malaria parasitaemia in their study of apparently healthy primary school children in Erunmn, a village in South West Nigeria. In their survey at Jos University Teaching Hospital Angyo *et al.*, (1996) reported that out of 2008 children admitted in the emergency paediatric Unit, 501 (25%) were admitted with diagnosis of acute severe malaria. It was found that malaria often occurs as a co-morbid condition with some other prevailing health problems. Uneke *et al*., (2005) investigated malaria and HIV co-infection in Jos-Plateau and reported that high prevalence of malaria parasitaemia existed in individuals infected with HIV. HIV induces immune suppression which leads to the emergence of opportunistic protozoa parasitic diseases that can increase malaria prevalence or exacerbate the disease manifestation. In a study of the prevalence of malaria parasite among pregnant women in Ihiala Local Government Area of Anambra state, South Eastern Nigeria, Yah *et al*., (2005) reported that malaria still remains a high prevalent parasitic infection among pregnant women with a stable transmission rate. Eneanya, (1998) in her study at Oji River in South-Eastern Nigeria noted a high prevalence of malaria among the local population with the highest rate of parasitaemia in the age group below 19 years.

In Ebonyi State South East of Nigeria, Ani (2004) investigated the endemicity of malaria among primary school children in the state and reported a high prevalent rate of 40.08%.This indicates a high degree of malaria parasitaemia among the study population and in the state in general. Ojukwu, (2002) also surveyed the pattern and outcome of paediatric malaria admissions in Ebonyi State and recorded 83.3% and 16.7% prevalent rates among the under 5s and those above 5 years respectively. He also noted that the severe forms of malaria were more common in under 5s and that severe anaemia was the most common clinical form of severe malaria. Children and infants are at risk of death from severe and recurrent acute attack of malaria because at that age, they had not acquired active immunity against the malaria parasite having lost the passive immunity they acquired from their immune mothers.

1.2.2 Malaria Burden

The burden of malaria is geographically biased towards sub-Saharan Africa. It is thus a health problem that has attracted concerted efforts over the years. It is quite unfortunate that despite these efforts it still remains one of the most public health problems and a leading cause of morbidity and mortality especially in sub-Sahara Africa. The sub- Saharan Africa is the most endemic region in the world and it is this region that malaria claims its highest toll. About 74% of the people in the region live in malaria endemic areas while 13% of them live in areas where there are periodic outbreaks of epidemics of the disease. Despite efforts to reduce transmission and increase treatment, there has been little change in which areas are at risk since 1992 (Hay *et al*., 2004). In many developing countries and in Africa in particular malaria exerts an enormous burden on man through medical costs and days of labour cost. The total days of labour cost coupled with costs of treatment and the high mortality associated with the disease make malaria one of the main factors retarding development in Africa (Mutero *et al.*,1998). By sapping people's health,

strength and productivity, it further marginalizes and impoverishes them (David, 2000). The burden of the disease can be evaluated using various indicators; morbidity, mortality, disability adjusted life years (DALYS), impact on health systems and socio-economic impact. Philippe *et al*., (2001) ranked malaria eleventh among overall causes of death, and eleventh highest on scale of disease burden measured by DALYs (See table 1).

Table 1: Malaria Mortality and Loss of DALYs in 1995

	Morality (1000s)	Mortality age 0-4 (1000s)	Clinical cases (1000s)	DALYs (1000)s
Total	1,110	793	272,925	39,267
Males	572	417	136,572	20,188
Females	538	376	136,532	19080
High-income countries	0	-	-	0
Low-income countries	1,110	-	272,925	39,267
Africa	961	745	237,647	34,506
Americas	4	0	2,043	130
Eastern Mediterranean	53	36	13,693	1,854
Europe	0	0	0	0
South East Asia	73	10	15,791	2,185
Western Pacific	20	2	3,751	591

Source: World Health Organization (1999).

Malaria is one of the top killer diseases in sub-Saharan Africa. In affected countries, as many as 3 in 10 hospital beds are occupied by victims of malaria (WHO, 1998). It causes more than one million death each year most of them among children under five years of age and pregnant women (Bouyou-Akotet *et al*., 2003; Newman *et al*., 2003). In highly endemic areas, malaria is responsible for about 30-50% of fever

cases, 30% of out-patient consultations and 10-15% hospital admissions (WHO, 2000a, Greenwood *et al.*, 2005). It is estimated that 50% of the population of the world has at least one episode of malaria each year (Afolabi *et el*., 1997). It is the commonest cause of out-patient hospital attendance in all age groups and the commonest cause of mortality in children. According to David (2000), malaria kills a child somewhere in the world every thirty seconds. The majority of these deaths occur in Africa. Malaria in pregnancy is a major cause of low birth weight and infant mortality (Topley, 1998). Low birth weight also has a serious impact on the survival and the health status of such children.

The social and economic consequences of malaria are very grave. It has a multi-layered impact on our society and development. Estimates of the economic burden of malaria typically, include; the cost of treatment, prevention, loss of work time, decreased productivity due to brain damage from cerebral malaria, loss of investment and tourism and economic losses associated with infant mortality and morbidity (Greenwood *et al*., 2005). The morbidity and mortality resulting from the disease severely hamper economic progress in endemic regions (Joy *et al*., 2003). The burden, in many cases, is estimated to be higher than 1% of a country's Gross Domestic Product (GDP). A comparison of average per capita GDP in 1995 adjusted to give parity of purchasing power between malariaous and non-malarious nations demonstrated a five- fold difference (Sachs and Melaney, 2002).

It is a major contributory factor to poverty and it affects the poor primarily thereby exacerbating inequities in health and impeding development. It keeps large numbers of adults from work. In Africa where malaria reaches its peak at harvest time and hits young adults especially hard, a single bout of the disease costs an estimated equivalent of 10 working days. The cost of malaria control and treatment drains African economies. Practically every family has a case of malaria every year. In the low-income families, medication costs as much as 25% of their annual income

(WHO 2000a). Endemic countries spend their scarce resources on buying drugs, nets and insecticides for both treatment and control of malaria. Malaria stricken family spends an average of over one quarter of its income on drugs for malaria treatment, as well as paying prevention costs on ITN and other insecticides thus suffering loss of income. According to WHO (1998), the direct and indirect costs of malaria in sub-Saharan Africa exceeded $2 billion in 1997.

It is a major cause of absenteeism in school children as they can suffer an average of six malaria bouts each year. Medical sources in Abidijan blamed some 60 percent of absentee cases in Ivorian schools on malaria (Annon, 2001). In this way, malaria seriously undermines the educational attainment of children in endemic areas. It reduces the rate of cognitive developments of children thereby enhancing their poor performance at school.

Malaria therefore is a major factor in Africa's high rate of infant and maternal mortality and of low productivity in farming and other works. It afflicts primarily the poor who tend to live in malaria endemic areas and in dwellings that offer little or no protection against mosquitoes.

1.2.3 Life Cycle of Malaria Parasite

The life cycle of all species of human malaria parasite is essentially the same (Fig1).It comprises an exogenous sexual phase (sporogony) with multiplication in certain female *Anopheles* mosquitoes and an endogenous asexual phase (schizogony) with multiplication in the vertebrate host. The later phase includes the development cycle in the red corpuscles in the blood (erthrocytic schizogony) and the phase taking place in the parenchyma cells in the liver (exo-erythrocytic schizogony) (Francis and Warrel, 1993, Ezeigbo, 2005).

1.2.3.1 Life cycle in man or vertebrate host

Pre-erythrocytic phase

Following the inoculation of sporozoites by the mosquito, there is a brief period of about one hour when the blood is infected. Later the sporozoites disappear from the blood. Many are destroyed by phagocytes but some enter the parenchyma cells of the liver (hepatocytes) directly or via the kupffer cells to undergo pre-ercythrocytic schizogony (Facer, 1994). This takes about 7-10 days causing no symptoms. The first generation of merozoites is called cryptozoites while the subsequent ones are known as metacryptozoites.

For *P. vivax* and *P. ovale,* development in the liver into a schizont is delayed in a proportion of invaded cells and in these hepatocytes the parasites will lie dormant as hypnozoites. Months or years later they will awaken and develop and cause the relapses of infection characteristics of these species. Each fully developed hepatic schizont of *P. vivax, P. ovale* and *P. malariae* liberates up to 15,000 merozoites into the host's blood stream whereas the hepatic schizont of *P. falciparum* contain 30,000 or more merozoites. Furthermore, the exoerythrocytic phase of development of *P. falciparum* takes average of 5-7 days (and may be shorter) compared to 7 days or longer in the benign malaria. This early amplification of the infection is greater in the potentially lethal *falciparum* malaria (NIAID,2012).

Later, the exo-erythrocytic merozoites invade the blood stream and penetrate the erythrocytes where they undergo schizogony. The trophozoite forms contain food vacuoles which displace the nucleus to one side like a signet ring. Hence this stage is referred to as the signet ring stage. Later the vacuole disappears and haemozoin pigment granules are deposited. This is segmenta the state. Eventually the red blood cells rupture to release the merozoites, haemozoin and other metabolic wastes into the blood stream. The rupture of parasitized cells is synchronized such that they all

rupture at definite intervals characteristic of each species. This periodically accounts for the bout fever at regular intervals caused by the metabolic wastes. The erythrocytic schizogony is repeated several times producing several generations of merozoites with the infection of new cells (Prescott *et al*., 2005). Some of the merozoites develop into gametocytes within the erythrocytes. No further development occurs until they are ingested by mosquito while it feeds.

1.2.3.2: Life Cycle in the Mosquito

When a female *Anopheles* mosquito ingests the blood of a person or animal with malaria parasite in the circulation, the asexual forms of the parasite are digested together with the red blood cells while the mature sexual cells (gametocytes) undergo further development(Long, 1999). In the stomach of the mosquito , the gametocytes develop into macrogamete and microgamete which are female and male gametes respectively after ex-flagellation. The macrogamete is fertilized by one microgamete to form a zygote (an ookinate). This penetrates the cell wall of the mosquito and lies between the epithelium and the basement membrane (Philips, 2001).

The oocyst nucleus divides several times with the first division being a reduction division to produce a number of sporoblasts. These divide further to produce thousands of haploid thread-like porozoites. When mature, the oocyst bursts releasing the sporozoites into the haemocoel of mosquitoes. Some of these sporozoites penetrate salivary gland of the mosquito and are injected with saliva into blood when the mosquito bites man to suck blood (see Figure 1).

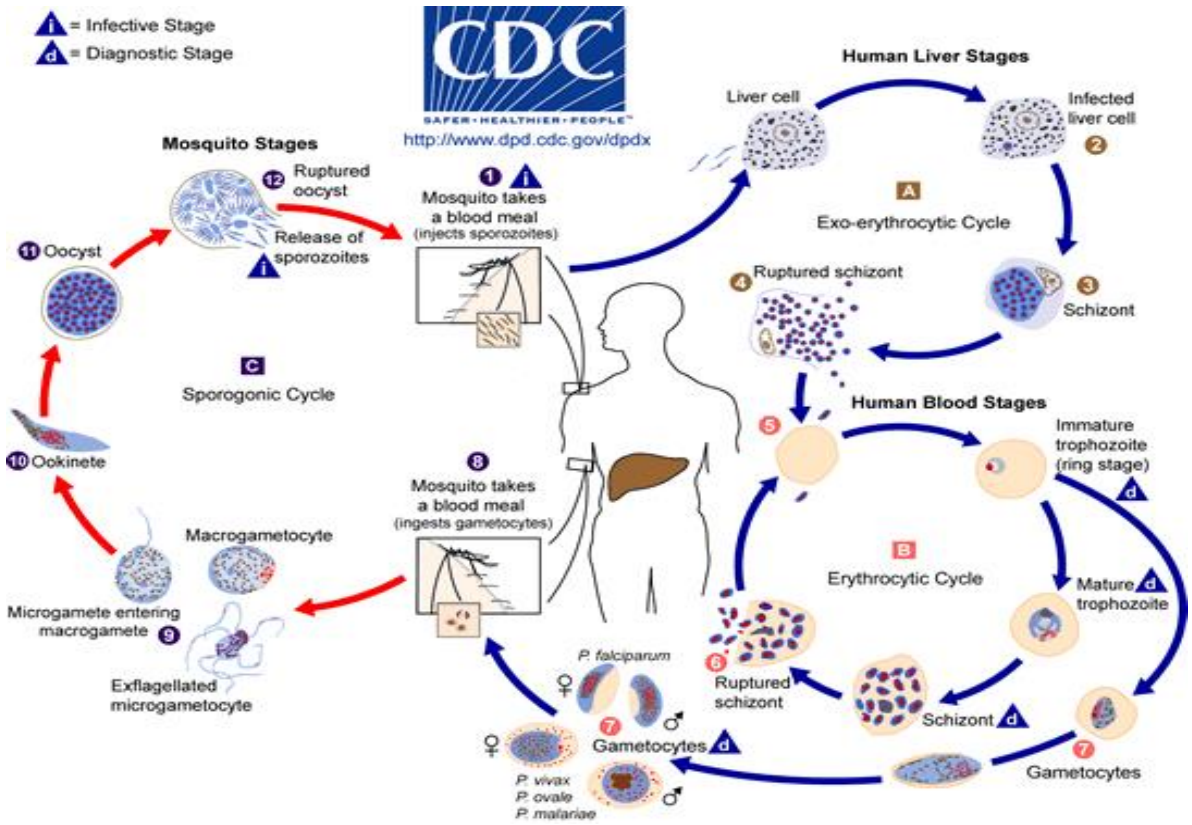

Figure 1: Life Cycle Malaria Parasite after Centre for Disease Control (2006)

1.2.4 Mode of Transmission

Malaria is transmitted by *Anopheles* mosquitoes, the number and type of which determine the extent of transmission of infection in a given area.

It is transmitted to humans by the bite of female *Anopheles* mosquitoes that are infected with protozoan parasite of the genus *Plasmodium.* The three most efficient species according to Azuike (1991) are *Anopheles gambiae, A. arabienses* and *A. funestus* . The transmission of malaria involves the transfer of the parasite from mosquitoes to man as much as from man to mosquitoes. The bite of an *Anopheles*

mosquito carrying sporozoites within its salivary glands involves three distinct but linked processes. These are the penetration and probing by the mouth parts into the skin, the injection of saliva containing sporozoites and blood feeding. Sporozoites injected into the skin during probing seem to be the only ones capable of reaching the liver of the host. Sporozoites injected into venous blood during feeding have no effect on the parasitic cycle as they are immediately re-ingested by the mosquito in its blood feed (Robert, 2001).

Transmission of malaria is affected by climate and geography and often coincides with the rainy season (WHO, 1998). It can be transmitted to man through three known ways which are (1) vector transmission (2) blood transmission and (3) congenital transmission.

1.2.4.1 Vector Transmission

Whereas many of the other human parasites seem happy to live in relative harmony with their hosts, those of *Plasmodia* have tendency to multiply rapidly and in an uncontrolled way in human hosts which may prove fatal. *Anopheles gambiae, Anopheles arabienses* and *Anopheles funestus* are the three most important species of *Anopheles* mosquitoes that are involved in malaria transmission (Federal Ministry of Health, 1990; Azuike, 1991). The disease is acquired when a biting and infected female *Anopheles* mosquito takes a blood meal with its proboscis in order to stimulate its reproduction. While probing for blood, she injects the microscopic parasite which then finds its way to the victim's liver. There it invades a few liver cells and develops over a period of one to two weeks during which the human host is blissfully unaware of the infection. At the end of this incubation period, the infected liver cells rupture and release a much increased number of parasites into the blood stream where they rapidly invade the circulating red blood cells. Once inside the red blood cell, the malaria parasite proceeds to consume the contents of its new home and to grow. At the end of two to three days, the destroyed cells burst

releasing more parasites which immediately invade more red blood cells. Thus the infection expands and within a week the victim begins to feel ill (White, 1991).

1.2.4.2 Blood Transfusion

Malaria can also be transmitted by transfusion of blood from an infected person to a healthy person. Induced malaria by blood transfusion was first reported in 1911 (Woolsey, 1991) and it is well established that all four human malaria parasites (*P. falciparum, P. vivax, P. ovale and P. malariae*) may be transfusion-transmitted (Wylie, 1993). Although blood is used in the management of emergencies involving patients with life-threatening illnesses as a life-saving venture, it also poses problems if not well managed. There are always a risk of immunological adverse reactions and transmission of other blood-borne pathogens (Cheesbrough, 1998). The transmission of malaria by blood transfusion is a serious risk, as the diagnosis of malaria in the recipient is unexpected and often missed (Kinde-Gazard *et al*., 2000). Hence, infected blood donors are considered a potential hazard for blood recipients. The fact that malaria parasites can survive in red blood cells at refrigerator temperatures (2-6^{o}C) for days or weeks led to the original exclusion of all blood donors who could represent a potential risk (Sazama, 1991). When such transmission takes place, a sexual blood form of the parasite continues to develop in the peripheral blood of the recipient but exo-erythrocytic stages are not formed in the liver. This is because these forms originate from sporozoites inoculated by mosquitoes themselves. Relapses do not occur and therefore malaria transmitted in this way is easily cured.

In affluent parts of the world such as Canada, USA and Switzerland where malaria has long been eradicated, regulations on blood transfusion criteria are already operational (Mungai *et al*., 2001; Frey-Wettstein *et al*., 2001). In most parts of sub-Saharan Africa where malaria scourge is still very severe, such regulations are essentially lacking, largely because of the dearth of scientific information on the risk of transfusion-transmitted malaria in many areas of intense or stable malaria transmission (Okocha *et al*., 2005). The possibility of acquiring malaria by heroin intravenous injection has also been established. Cases of induced malaria in narcotic abuses due to *falciparum* infection were reported in Europe by the World Health Organization (WHO, 1993).

1.2.4.3 Congenital Transmission

Congenital malaria is the uterine transfer of malaria from mother to child in the womb. It is now recognized that malaria parasites can cross the placental barriers and enter fetal circulation and give rise to congenital malaria (Brabin, 1991). Vertical transmission of malaria across the placenta from mother to feotus is diagnosed when parasitaemia is found in the neonate within 7 days of birth, or later if there is no possibility of postpartum infection by mosquito bite or blood transfusion.

According to Warrel, (1993), all four species of *Plasmodium* can produce congenital infection but *Plasmodium falciparum* and *Plasmodium vivax* are most common. Such infection results to low birth weight, abortion, still birth or death in new born babies. In endemic regions, the incidence of congenital malaria is usually extremely low or it may not result in any significant problem despite the high prevalence of placental infection. This is due to the fact that sufficient antibodies would have also crossed from maternal compartment to the fetal compartment to protect the new born babies in the early phase of life (Okonofua, 2001). The incidence is much higher

in infants born to non-immune mothers and increases during malaria epidemics. Outside the endemic areas, *Plasmodium* malaria causes a disproportionate number of cases because of its very long persistence.

1.2.5 Symptoms of Malaria

The symptoms of malaria depend very much on the amount of pervious exposure to the disease and thus on the level of background immunity (White 1991). In many parts of the tropics especially in Africa, transmission of falciparum malaria is intense and everyone is infected repeatedly. Children may get severe malaria in the first few years of life but gradually, with repeated infections most of them develop immunity. By the time they are adult they no longer show malaria symptoms as they did earlier. This hard-fought-for immunity of the survivors of malaria is easily lost if the subjects leave a malarious area. In other areas of the world where transmission of the disease is less intense, symptoms develop at any age.

Malaria patients usually present with anorexia, teeth-chattering chills, high fever which occur at exactly the same time every one or two days. Others are joint weakness, hepatosplenomegaly, coke coloured urine and in some cases vomiting and diarrhea. Headache, dizziness and general body pains are also clinical symptoms of malaria (Eneanya, 1998). There may be the feeling of tingling in the skin, particularly with malaria caused by *P. falciparum.* The classical symptom of malaria is cyclical occurrence of sudden coldness followed by rigor, then fever and sweating lasting 4-6 hours occurring every two days in *P. vivax* and *P. ovale* infections and every three days for *P. malariae. P. falciparum* can have recurrent fever every 36-48 hours or less pronounced and almost continuous fever. For reasons that are poorly understood, but which may be related to high intracranial pressure, children with

malaria frequently exhibit abnormal posturing, a sign indicating severe brain damage (Idro *et al*., 2007).

1.2.6 Diagnosis

Diagnosis of malaria with certainty is on identification of malaria parasite in blood films of patients together with other symptoms associated with the disease. Diagnosis is parasitological and is made by examining thick blood films stained with field or Giemsa stains. However, in *P. falciparum* infection the parasite-containing erythrocytes tend to stick to the capillary endothelium and block the capillaries and thus the circulation. This reduces the number of parasites found in the blood stream and may lead to misdiagnosis or under estimation of the severity of the disease and its complications (Ouali *et al*.,1996). Although species diagnosis can be made on thick films, it is also usually made on thin films stained with Leishman or Giemsa stains. This depends on the characteristics of the trophozoites, schizonts and gametocytes. As a rule only ring forms and gametocytes are found in the peripheral blood in *falciparum* malaria unless the infection is severe, in which case, schizonts also appear (Azuike, 1991). Due to the large and increasing number of children attending the dispensaries, clinics and out-patients department of hospitals in many areas where the disease is endemic, it is not always possible to investigate every suspected case even where sufficient laboratory staff is available. Hence a diagnosis of clinical malaria is commonly made in children who present with fever without other overt childhood diseases and chloroquine is often given as a therapeutic measure.

Zaman (1972) used impregnated filter paper strips for staining malaria parasites; while Janis (1971) described a technique based on the fact that acridine orange selectively stains nucleic acid. Sadum (1972) also reviewed the research and

development of serological test for malaria while Eden (1973) looked into the feasibility of computer screening of blood films for the detection of malaria parasite. A more recent diagnostic method in the fight against malaria is the Quantitative Buffy Coat method of detecting blood parasites. This method depends on centrifugation of a blood sample in acridine-orange coated capillary tubes. The centrifugal stratification of *Plasmodium* parasites greatly enhances their visibility by concentrating them enabling extremely rapid and highly sensitive detection and identification. Oloo *et al*.,(1994) evaluated the use of the Quantitative Buffy Coat method of detecting malaria infection in field surveys and reported that the QBC is quicker, with high sensitivity and will prove useful in clinical and epidemiological screening, especially when parasitaemia is low. The ability of the QBC to detect lower levels of infection enables health workers to diagnose and treat the disease earlier in its course. The extraordinary sensitivities of the QBC malaria test ensures that patients who might otherwise be inaccurately diagnosed as malaria-free using thick film method will not be released from treatment only to become transmission sources for families and neighbours. In areas where microscopy is not feasible antigen detection tests implored (Pattanasin *et al*., 2003). Optimals – IT® reliably detects falciparum down to 0.0% parasitaemia and non falciparum down to 0.1%. Paracheck – PF® detects parasitaemia down to 0.002% but will not distinguish between falciparum and non-falciparum malaria (Pattanasin *et al*., 2003).

Another recent diagnostic technique in the fight against malaria is Polymerase Chain Reaction (PCR). The technique is more accurate than microscopy but it is expensive and require trained and specialized staff. Areas that cannot afford even simple laboratory diagnostic tests often use a history of subjective fever as the indication to treat for malaria.

1.2.7 Socio-Cultural Beliefs on Malaria

Most rural dwellers of malaria endemic areas are not cognizant of the fact that mosquitoes transmit malaria parasites which they cannot see with their unaided eyes. It is usually unimaginable for them that these parasites cause fever or the breakdown of parts of their blood. The findings of Munguti (1998) in Baringo district Kenya revealed that the IIchamus believe malaria to have multiple causes. These include “nkonjong” (mosquito), “kure nariewa” (fresh milk) and “nkare tarumo” (dirty water). The same beliefs are shared by the Jugen which believe that “check che makiyo” (fresh unboiled milk) could cause malaria. People also believe that malaria could be caused by wild vegetable such as *Solanium gynandia.* Further ethnographic studies by Munguti (1998) revealed that the co-relation between milk and malaria is logically valid, especially in semi arid areas such as Marigat where milk is plentiful during and immediately after the rainy season. This coincides with the period that mosquito population increases and consequently the incidence of malaria. Similar findings have been reported in Ghana and Gambia (Agyepong, 1992, Atkins *et al*.,1995).

In Nigeria, there is a belief among the rural dwellers of Ohaozara in Ebonyi State that malaria is caused by the consumption of fresh and boiled groundnuts, fresh corn and pears (Oral personal comminication). Hence they advocate frying of fresh groundnuts rather than boiling them. These food items are plentiful during the rainy season which is also the time that mosquito populations are on the increase which leads to high incidence of malaria. The people also believe that consumption of oily food and working for long hours under the sun can cause malaria. This is in keeping with the result of a research carried out by Afolabi (1996) in an isolated community on the Atlantic Coast of Lagos. According to him the community considers malaria as being caused by consumption of oily food or drinking bad water. Therefore a woman would fret at her child playing in the sun but not at the same child going about or sleeping naked at night.

1.2.8 Pathological Effects of Malaria

The developing trophozoite of malaria parasite depends upon the host for its nutritional requirements. It follows therefore that the development of the parasite must in part depend on successful competition with the host for certain substances required equally by both. The pre-erythrocytic state of infection produces minimal histopathological changes and absolutely no detectable symptoms or functional disturbances in the host. Pathological processes in malaria are the results of the erythrocytic cycle (CDC, 2006). Pathology with all malaria species is related to the rupture of infected erythrocytes and the release of parasite materials and metabolites, hemozoin and cellular debris. In addition to other known paroxysms, the deposition of hemozoin has long been known as a characteristic feature of malaria. There is an increase activity of the reticulo-endothelial system, particularly in the liver and spleen and thus their enlargement as evidenced by macrophages with digested infected and normal erythrocytes and hemozoin. Except for *P. falciparum*, the pathology associated with malaria tends to be rather benign.

1.2.8.1 Host Erythrocyte Modification

Once the parasite is inside the erythrocyte, it undergoes a trophic phase followed by replicating phase. During this period, the parasite modifies the body of the host to make it a more suitable habitat. For example, the erythrocyte membrane becomes more permeable to small molecular weight metabolites reflecting the need of an actively growing parasite. Another modification is the cytoadherence of *P. falciparum*-infected erythrocytes to endothelial cells which results in sequestration of the mature parasites in capillaries and post capillary venules. This sequestration leads to microcirculatory alterations and metabolic dysfunctions which could be

responsible for many manifestations of severe falciparum malaria. The cytoadherence to endothelia cells confers at least two advantages for the parasite which are 1) a micro aerophilic environment which is better suited for parasite metabolism and 2) avoidance of the spleen and subsequent destruction.

1.2.8.2 Cytoadherence and knobs

A major structural alteration of the host erythrocyte is electron-dense protrusions or knobs on the erythrocyte membrane of *P. falciparum* infected cells. The knobs are induced by the parasite and several parasite proteins are associated with them. Two proteins which participate in knob formation or affect the host erythrocyte sub-membrane cytoskeleton and indirectly induce knob formation are the knob-associated histidine-rich protein (KAHRP) and erythrocyte membrane protein-2 (PfEMP2), also known as MESA. The knobs are believed to play a role in the sequestration of infected erythrocytes since they are points of contact between the infected erythrocytes and vascular endothelial cells and parasite species which express knobs exhibit the highest levels of sequestration. In addition, disruption of the KAHRP results in loss of knobs and the ability to cytoadhere under flow conditions. Other proposed cyto-adherence lignds include a modified band-3, known as pfalhesin sequestrin, rifins and clag 9.

1.2.8.3 Endothelial cell Receptors

Several possible endothelial receptors have been identified by testing the ability of infected erythrocytes to bind in static adherence assays (Beeson and Brown, 2002). One of the best characterized among these is CD 36. Infected erythrocytes from most parasite isolates bind to CD 36 and binding domains has been mapped to the CIDR of PfEMPI. Rosetting is another adhesive phenomenon exhibited by *P. falciparum* – infected erythrocytes. Infected erythrocytes from some parasite

isolates will bind multiple uninfected erythrocytes and PfEMPI appears to have a role in at least some rosetting. Possible receptors include complement receptor – (CRI) blood group A antigen, or glycosaminoglycan moieties on an unidentified protecoglycan.

1.2.8.4 Antigenic Variation

The encoding of the cyto-adherence ligand by a highly polymorphic gene family presents a paradox in that receptor/ligand interactions are generally considered highly specific. Selection for different cyto-adherent phenotypes result in a concomitant change in the surface antigenic type. The expression of a particular PfEMPI will therefore result in a parasite with a distinct cyto-adherent phenotype and may affect its pathogenesis. For example, Costa *et al*., (2003) reported a higher proportion of isolates which bind to CSA from the placenta as compared to the peripheral circulation of either pregnant women or children. Similarly, the association of PfEMPI with rosetting also explains the variability of the rosetting phenotype. Although sequestration offers many advantages to the parasite, the expression of antigens on the surface of the infected erythrocyte provides a target for the host immune system. The parasite, however, counters the host immune response by expressing antigenically distinct PfEMP molecules on the erythrocyte surface. This allows the parasite to avoid clearance by the host immune system, but yet maintain the cyto-adherent phenotype. According to Robert (1992), this antigenic switching may occur as frequently as 2% per generation in the absence of immune pressure.

1.2.8.5 Human Genetics and Innate Resistance to Malaria

Certain genetic diseases and polymorphisms have been associated with decrease in malaria infection or disease. For example, individuals that lack the Duffy blood group antigen are refractory to *P. vivax*. A large proportion of the populations in

Western Africa are Duffy negative and that is why *P. vivax* is rarely found in West Africa. This innate resistance led to the identification of the Duffy antigen as the erythrocyte receptor for merozoite invasion. The glycophorin receptors which *P. vivax* needs to attack and invade red cells are missing on Duffy negative red cells. In Southeast Asia and the pacific Islands, ovalcytosis which is due to a mutation in an erythrocyte membrane protein called band 3 occur causing the cells to have oval shape. The mutation causes the erythrocyte membrane to become more rigid and more refractory to merozoite invasion. Ovalcytosis causes chronic anaemia but protects people from developing cerebral malaria as persons with it have lower densities of *P. falciparum* and *P. vivax.* Glucose-6-phosphate dehydrogenase (G6PD) deficient erythrocytes would have additional oxidants produced as a result of parasite metabolism and the digestion of haemoglobin may overwhelm the infected erythrocyte and lead to its destruction before the parasite is able to complete its schizogony. In the same way, possession of certain human leucocyte antigen (HLA), B-thalassaemia and S-thalassaemia genes protect against severe malaria. In sickle cell disease, there is a mutation in the HBB gene which encodes the betaglobin subunit of haemoglobin. The normal allele encodes a glutamate at position six of the beta globin protein while the sickle cell encodes valine. This change from hydrophilic to hydrophobic amino acid encourages binding between haemoglobin molecules with polymerization of haemoglobin deforming red blood cells into a ‘sickle’ shape. Such deformed cells are cleared rapidly from circulation mainly in the spleen for destruction and recycling (Ike and Odikamnoro,2005). In the merozoite stage of its life cycle the malaria parasite lives inside red blood cells. Infected cells normally survive until the parasite reproduces but if the red cell contains a mixture of sickle and normal haemoglobin, it is likely to become deformed and be destroyed before the daughter parasites emerge. Thus, individuals heterogzygous for the mutated allele known as sickle cell trait (AS) may have a greatly reduced chance of serious malaria infection. This is a classic example of heterozygous advantage.

1.2.8.6 Organ pathology

In *P. falciparum* malaria, severe and life-threatening conditions commonly arise. These cause dysfunction of vital organs such as the lungs, spleen, kidneys, liver and most famously the brain during cerebral malaria. Severe anaemia can also occur. These are associated with most of the mortality of acute malaria. Chronic infection with *P. malariae* can also result in a nephritic syndrome and this too can eventually be fatal.

Kidney

Malaria infections repeatedly have been reported to induce nephritic syndrome and acute renal failure in different parts of the world (Barsoum, 2000; Mehta *et al*, 2001; Mishra, *et al*, 2002; Koh *et al*., 2006; Das, 2008 and Hanson *et al*., 2010). Both children and adults living in areas endemic for malaria infection are affected by malaria-induced nephrotic syndrome. The pathological lesions seen in the kidneys in malaria are variable. Non immunes have a higher risk of developing acute renal failure than the semi-immunes and children with cerebral malaria have higher rates and more severe course of acute renal failure than children with mild malaria. In cerebral malaria, the striking features is gross congestion of the vessels with parasitized erythrocytes especially in the capillaries of the glomerular tuft. Falciparum malaria results in an acute and transient self-limiting glomerulonephritis whereas *P. malaria* infection leads to nephritic syndrome. This type of malaria is associated with morbidity and mortality in the high risk groups. Risk factors for high mortality include late referral; short acute illness; high parasitaemia and presentation with oliguria, hypotension, severe anaemia or significant jaundice. Patients with severe diarrhea, multisystem involvement, hepatitis or acute respiratory distress also have a grave prognosis. In children, younger age and the

absence of splenomegaly are associated with higher mortality. Opportunistic pulmonary viral or bacterial infections may also be encountered in patients with malaria acute renal failure, increasing the risk of mortality. Serious cases of renal problems associated with malaria take the form of nephrotic syndrome, which gradually progress to renal failure if not managed promptly (Edward and Boucher, 1991; Stevens *et al*., 2006; Ogbadoyi and Gabi, 2007). It is characterized by severe proteinuria, rise in blood urea, low urine specific gravity, low ratio of urinary to blood urea and hyperkalaemia and metabolic acidosis (Mishra *et al*, 2002). Acute renal failure as a serious complication of malaria carries a high reported mortality of 15-45% in India (Barsoun, 2000). This depends to a large extent on the local prevalence of malaria and on the pattern of referral to a specialized centre for diagnosis, and subsequent treatment.

Acute tubular necrosis (ATN) which is the most common pathology result from hypovolaemia, peripheral pooling of blood, blockage of microcirculation by parasitized red blood cells are non-specific effects of infection (Mishra *et al*, 2002; Naqvi, *et al, 2003).* Massive intravascular haemolysis in acute renal failure also occurs in patients with glucose-6-phosphate dehydrogenase deficiency. In developing countries, limited medical resources at primary health care centers and late referrals compound these outcomes. However, this is usually reversible with appropriate management. In chronic stages, malaria effects on kidneys will lead to loss of cortico-medullary differentiation in ultrasound scan (Sutton, 1998). In Black water fever and in patients with inherited erythrocyte enzyme defects such as glucose -6- phosphate dehydrogenase deficiency who have been given oxidant drugs, large amounts of hemoglobin are cleared by the kidney following intra vascular haemolysis. About one-third of non-immune adults with severe *P. falciparum* malaria will show biochemical evidence of renal dysfunction. This is less common in children and particularly rare in African children (Francis and Warrel, 1993).

Nephrotic syndrome which may lead to acute renal failure in malaria needs urgent recognition and management. Multiple complications need urgent management in a tertiary care hospital with multidisciplinary approach.

1.2.9 Epidemiology of Malarial Renal Impairment

Malaria is widely spread throughout the world and affects close to 400 million people, most of whom live in Africa, India, Southeast Asia and Latin America (Barsoum, 2000). Acute renal failure is a frequent and serious complication of falciparum malaria in non-immune adults and older children (Eiam-Ong, 2003). Its proportion in complicated falciparum malaria is reported to be several folds higher in patients from non-malarious regions than those from high malaria transmission areas. Its incidence is also reported to be as high as that of cerebral malaria (Weber *et al*; 1991). A large proportion of malaria acute renal failure admitted to a hospital in Ethiopia consisted of non-immune visitors (Habte, 1990). Occurrence of renal impairment in severe malaria is quite common in Southeast Asia and India subcontinent where intensity of malaria transmission is usually low with occasional micro foci of intense transmission. High incidence of malarial renal impairment has also been reported from Vietnam (Tran *et al*., 1992). Severe malaria in this part of the world is usually a multi-system disease where more than 40% of severe malaria patients had acute renal failure and more than 55% of fatal cases had acute renal failure on admission that rose to 70% by the time they died (Tran *et al*., 1992). Hypercatabolic acute renal failure associated with cerebral malaria, heavy parasitaemia and hyperbilirubinemia was noticed in Malaysian patients. It constituted 23.3% of severe malaria patients in a hospitalized study by Jegasothy *et al*.,(1994).

A significant increase in the incidence of malarial renal impairment has been reported from several centers across India where complications of *P. falciparum* malaria occur in all age groups. A change in the clinical presentation of severe malaria in India has shifted from single to multiple complications. Mortality in cerebral malaria patients with associated complications such as jaundice and acute renal failure is twice as high as that with cerebral malaria alone. In a study by Panda *et al*., (2003) from southern part of Orissa State in eastern India, it was reported that 35% of severe *P. falciparum* malaria cases had acute renal failure. A similar report from an intensive care unit of a tertiary care university hospital also found malaria as an important cause of multiorgan failure in India and regardless of the organ system involved mortality was significantly higher with multiple organ failure than with one or no organ failure. Malaria has therefore become the emerging cause of renal impairment in the country India (Krishnan and Karnad, 2003).

Studies from Pakistan also reported malarial renal impairment contributing significantly to total renal impairment burdens in that country (Abdul Manan *et al*., 2006). In recent years, malaria-induced renal damage with associated morbidity and mortality has been reported more frequently in semi-immune African children (Zewdu, 1994; Waisu and Kayode, 2004). Weber *et al*., (1991) studied renal involvement in Gambian children with cerebral or mild malaria and reported that renal involvement with malaria is common in the Gambia with pre-renal glomerular and tubulo-interstitial factors contributing. According to them, it is more pronounced in children suffering from cerebral malaria than those with mild malaria.

In Congo, acute renal failure is a common problem. Assounga *et al*., (2000) carried out a study on the aetiology and outcome of acute renal failure in Congo-Brazzaville. According to their report, the main aetiologies of acute renal failure included acute gastroenteritis with dehydration (25.7%), nephritic syndrome (14.7%), sepsis

(15.23%), malaria (12.38%) and acute glomerulanephritis (9.5%). This shows that malaria was one of the leading infections that caused acute renal failure in their study. The outcome was recovery in 50.5%, death in 37% and chronic renal failure in 12.5% of cases.

In Nigeria, some studies though few have been carried out to assess the involvement of malaria in the renal function of humans. Ekeanyanwu *et al*; 2010 assessed renal function of *Plasmodium falciparum* infected children and they reported that there is a form of renal impairment associated with malaria infection in children. Sowunmi (1996) also assessed renal function in 40 children during the acute illness and after recovery from falciparum malarial in Ibadan. His report suggested that acute intrinsic renal impairment occurs during apparently uncomplicated falciparum malaria in children. Ogbadoyi and Tsado (2009) worked on the renal and hepatic dysfunction in malaria pateitns in Minna, North Central Nigeria and reported that renal dysfunction, acute renal failure and liver dysfunction are clinical features of malaria in Minna.

1.2.10 Pathogenesis of malarial renal impairment

Malaria renal impairment can occur as an isolated complication or as a component of maltiorgan failure. Though precise mechanism of renal failure in falciparum malaria is not clearly known, several hypotheses have been proposed. Such hypotheses include mechanical obstruction by infected erythrocytes, immune mediated glomerular pathology, fluid loss due to multiple mechanisms and alternations in the renal microcirculation (Eiam-Ong and Sitprija, 1998). Cytoadherence of *P. falciparum* infected red blood cells (IRBSC) to the vascular

endothelial cells of different host organs along with rosette formation is considered as a most important mechanism of severe malaria. Infected red blood cells preferentially sequester in the deep vascular beds of vital organs such as the brain, liver, lung, spleen, intestine and kidney. Parasite proteins known as variant surface antigens which are expressed on the surface of infected red blood cell also mediate adhesion of such cells to host vascular endothelial receptors. Therefore more infected red blood cells are seen in renal vasculature of malaria patients with acute renal failure than those without acute renal failure.

Recent studies have also shown mononuclear cells in glomerular and peritubular capillaries (Nguansangiam *et al*., 2007; Patnaik *et al*., 1994). Activation of such mononuclear cells with glomerular and peritubular capillaries is likely to induce host immune reactions by releasing cytokines, reactive oxygen intermediates (ROI) and nitric oxide (NO) locally. Exaggerated host immune response is considered an important mechanism for several complications in malaria. Host-parasite interaction may result in mechanical, immunological and humoral responses which while eliminating parasites also injures host tissues. Restricted local blood flow in the kidneys is considered a major contributor for malarial renal impairment. Low intake of fluids, loss of fluids because of vomiting and pyrexial sweating may be responsible for dehydration and renal ischemia. A generalized vasodilation with an associated decease in systemic vascular resistance is also considered an important contributor for septic shock as well as malaria acute renal failure.

Low intake of fluids, loss of fluids because of vomiting and pyrexial sweating, cytokine and NO mediated arterial vasodilation specifically organ specific release of NO, resistance to vasoactive hormones, cytopathic hypoxia leading to decreased ATP synthesis, cytoadherence of parasitized red blood cells etc all may contribute singly or in combination towards malarial acute renal failure. Pathogenesis of malarial renal impairment occurs in two forms which are:

1) Mild cases – here, there is not much change in renal parenchyma, there may be minimal tubular degeneration, mild renal parenchymal change and presence of vacuoles.
2) Severe cases – there is tubular degeneration with distal tubular necrosis, proximal tubules are often loaded with malaria pigments, and haemoglobin granules may be seen in the tubular cells.

Malarial renal impairment is mediated through several mechanisms such as:

- Effect of parasitized red blood cells on microcirculation. Knob-like processes are formed on surfaces of parasitized red blood cells which help them anchor on the endothelium.
- Cytoadherence occurs due to thrombospondin formation from vascular endothelium. This is specific of *Plasnodium falciparum*.
- Loss of deformability of parasitized red blood cells occurs according to need of microcirculation. This leads to sluggish circulation and resultant renal ischemia.
- Hypovolumia may occur due to fever, sweating, decreased intake of fluid and vomiting.
- There may be release of chemical mediators such as Tumor necrosis factors-α, cachectin, cytokines, interleukins etc. This causes vasoconstriction, increased vascular permeability, catecholamine release, shock and tubular necrosis.
- Plasma viscosity is increased due to infection.
- Hyperbilirubinaemia occurs due to hemolysis. Black water fever in G6PD deficiency patients is also associated with renal impairment in malaria.

1.2.11 Clinical Presentation of malarial renal impairment

Clinical presentation of malarial renal impairment comes in two forms:

a) Acute renal failure as a component of multiorgan failure associated with anaemia, jaundice, hypoglycemia, acidosis or coma (Nally, 2002).

b) It could also present as a sole complication at a later stage when other complications have subsided with treatment.

In both cases, diagnosis is done by carrying out biochemical tests such as blood urea nitrogen, electrolytes, blood sugar. It is suspected when urine output <400ml /24/hrs and confirmed when serum creatinine <3gm/dl. Renal involvement in falciparum malaria can present as electrolyte abnormality, abnormal urinary sediments and increased urinary protein excretion, acute renal failure etc. Acute renal failure is usually associated with oliguria and in severe cases anuria with normal urination or polyuric. Oliguric phase varies from few days to weeks. Pre-renal azotemia presents with signs of dehydration. Malaria acute renal failure is catabolic in type characterized by rapid rise of plasma urea and creatinine due to increased catabolism. Sub-clinical impairment of renal function is also observed in a significant proportion of malaria pateitns. The common predisposing factors for malaria acute renal failure are volume depletion, gastrointestinal bleed, sepsis, nephrotoxic drugs etc. Mortality is higher when plasma creatinine at presentation is high, urine output is low, delayed referral to the hospital, and when associated with other complications (Sheiban, 1999). Malarial renal impairment patients have higher incidence of anaemia, jaundice, hypoglycaemia and prolonged coma. Acidosis appears early and may be associated with clouding sensorium, convulsions and coma.

Association of renal impairment has been reported from southeast Asia and Indian sub continent where malarial acute renal failure is common (Mishra *et al*., 2007). About half of all adult patients with cerebral malaria had biochemical evidence of renal impairment (raised blood urea and serum creatinine). The vulnerable groups of patients include pregnant women, patients with high parasitaemia, those with deep jaundice, those with prolonged dehydration and patients receiving NSAIDS.

1.2.12 Management of Malarial Renal Impairment

Malarial acute renal failure is suspected when urinary output falls to less than 400ml in 24hrs or 20ml/hr, which fails to improve after adequate hydration. Occasionally, it may be non-oliguric and be diagnosed by biochemical investigations. The diagnosis is confirmed when serum creatinine exceeds 3mg/dl (260umol/l) in adults and 1.5mg/dl (130umol/l) in children. For appropriate management of renal impairment in malaria, it is essential to distinguish between pre-renal azotemia and established acute renal failure. A simple way to do this is the measurement of urine specific gravity(Kellum *et al*., 2005). In pre-renal azotemia it is more than 1.02 while in established acute renal failure it is less than 1.01. The mainstay of management in malaria renal impairment revolves around

1) appropriate antimalarial therapy
2) fluid replacement (Phu *et al*., 2002)
3) renal replacement therapy
4) supportive therapy and
5) avoidance of nephrotoxic drugs.

Chroloquine is cheap, safe and effective in chloroquine sensitive parasites but it is no longer used for treatment of severe falciparum malaria because of widespread resistance of *P. falciparum* parasites to the drug. The current practice is to treat all patients of severe malaria including renal impairment with quinine or appropriate artemisinin derivatives through parenteral route (Dondrop *et al*., 2005; Dondrop and Day, 2007).

Preservation of renal blood flow and perfusion pressure prevents deterioration of renal function. This is achieved by infusion of fluids. Fluids are administered slowly and titrated against jugular venous pressure and urine output because acute renal

failure patients are vulnerable to post-transfusional volume overload. Close monitoring for signs of volume overload during transfusion of blood or fluid is therefore highly essential. Malarial renal impairment is hypercatabolic. Therefore hemodialysis or peritoneal dialysis is performed in conditions of rapid increase in creatinine concentration. Continuous removal of fluid and waste products minimizes the problems of fluid overlaod. In areas where malaria is endemic, peritoneal dialysis is usually the only available dialysis modality.

Diurectics are often used to increase urine output in oligura patients at risk of acute renal failure. Vasoactive drugs are often administered to improve either cardiac output or mean arterial pressure in the hope that renal blood flow will also be improved and renal protection achieved. Such drugs as Furosemide and dopamine are the drugs of choice. If high doses of dopamine fail to restore perfusion pressure, norepinephrine can be of high value. Nephrotoxic drugs such as ACE inhibitors, NSAIDs, aminoglycosides and cephalosporins should be avoided.

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1 Study Area

The study was a community based cross sectional survey. The study areas were villages in Isu Okoma Community in Onicha Local Government Area of Ebonyi State, South-eastern Nigeria. A government owned General Hospital is the largest health institution located in the area. There are also some comprehensive health centres that operate under the supervision of medical doctors. There is also a strong belief in and use of traditional medicine in the area. It is bounded to the West by Enugu State, to the South by Ohaozara, to the North by Ishielu and to the East by Ezza North Local Government Areas of the State. It is about 120-180 meters above sea level and covers a land area of approximately 51km^2. The study area is defined by

longitude $8^{o}6^{1}$ 6" E and latitude 6^{o} 22^{1} 28" N. The vegetation is characteristic of derived savannah with high rainfall intensity, high run-off volumes and high relative humidity and an average rainfall of about 1600mm-2000mm per annum (Mgbada, 2004). The mean daily maximum and minimum temperatures are 32^{o}C and 25^{o}C respectively. The natives/residents are prominently farmers but also engage in trading and crafts as well as public and civil services. Agriculture provides productive employment to over 85% of the residents and the main agricultural products include rice, yam and cassava. However, agricultural production is still rudimentary as the same local tools, old cultural practices and farming systems used over the ages are still in vogue. Such poor agricultural practices have been responsible for a high percentage of loss of forest resources and predisposition of the top soil to erosion.

There are two distinct seasons, the wet and the dry seasons, the former taking place between April and October, while the latter occurs from November to March. However, there could be occasioned rains in January, February and March. The dry season is also punctuated by a brief period of dry and dusty haze, known as harmattan. Malaria transmission is perennial in the area.

Figure 2: Map of Ebonyi State showing the Study Area. Ebonyi State Website

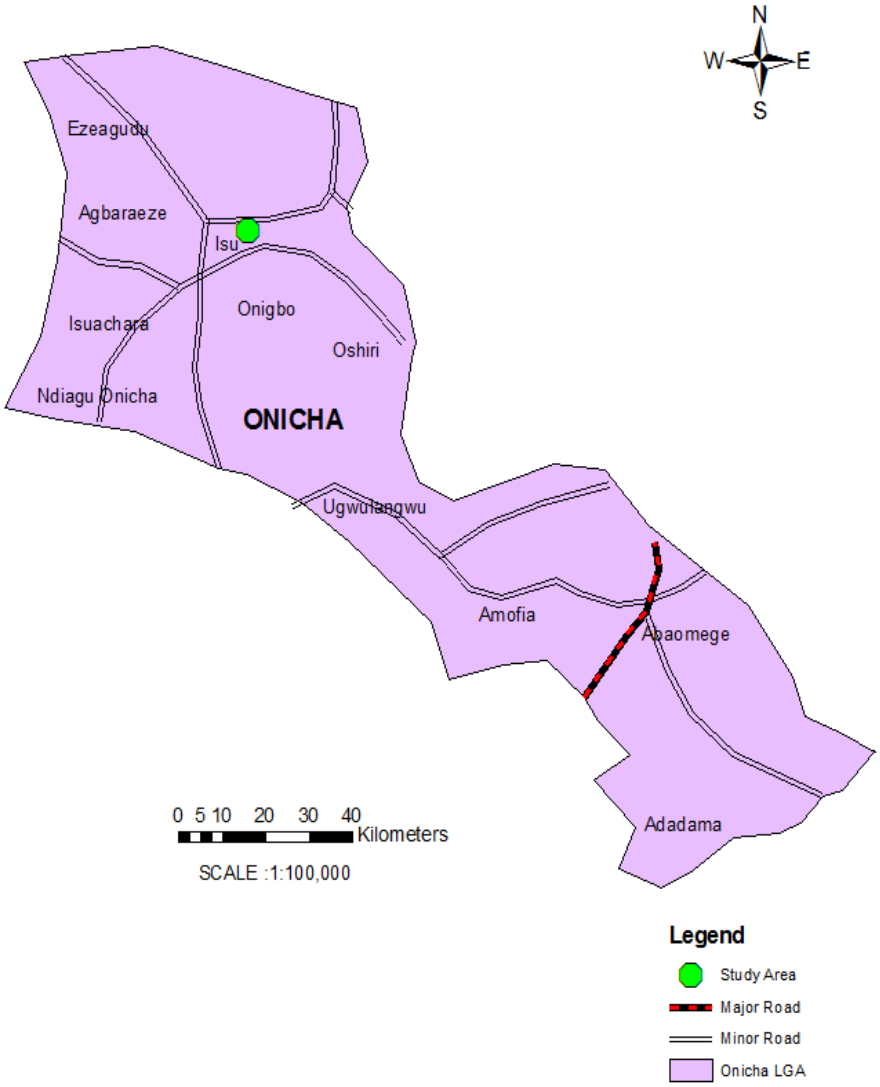

Figure 3; Map of Onicha L G A showing the study area.

2.2 **Methodology**:

Ethical consideration: Clearance was obtained from the Ethical Committee of the Federal Medical Centre Abakaliki, Ebonyi State. Informed consent of the village heads of the villages and those of the subjects involved in the research were sort and permission granted before the study commenced.

From seven villages that make up the community, a multi-stage sampling technique was used to sample three villages. A three-stage sample design was adopted in the survey of which selection of villages constituted the first stage/primary Sampling Units(PSUs), while selection of Households (HHs) formed the second stage/Secondary Sampling Units. This produced 40 households per village, out of which ,240 individuals (males and females) of ages not less than 3 years were randomly sampled. Thick blood smears of venous blood stained with Giemsa were examined microscopically for malaria parasitaemia (MP) and intensity. Biochemical parameters(urea, creatinine and albumin), electrolytes (sodium, chloride, potassium and bicarbonate ions) and haematological indices (packed cell volume, total leukocyte count and the differentials) of MP positive and negative individuals were determined using standard procedures. Urine samples of the same individuals were also collected for estimation of total protein levels (TP), specific gravity (SG) and pH. Ultrasonographic examination of kidneys was done to compare the features and sizes of both MP positive and negative individuals. All individuals not positive for malaria parasite served as controls. The subjects under study were provided with Insecticide Treated Nets (INTs) under which they slept throughout the study period. Those positive for malaria parasite were treated with current anti-malaria drugs (Lumartem, 3 tablets twice daily for 3 days) as prescribed by a medical doctor. The

level of malaria parasite clearance was noted after 3 weeks by a second malaria tests. The afore mentioned investigations were repeated after the malaria treatment and second malaria tests of malaria positive subjects. This is to investigate the possible involvement of malaria parasites in renal problems if any.

2.2.1 Investigation of blood samples

Venous blood was collected from each subject by a qualified nurse into EDTA bottles and the age, sex and number of the subjects marked carefully on the bottles using masking tape. Slides were also labeled and the number of the subjects marked on them. Thick blood films were smeared on clean dry slides for malaria parasite (MP) identification. They were air-dried before being packed in slide racks. The bottles containing the blood samples were properly corked, inspected for labels and the whole arrangement transported to the laboratory for analysis. For malaria parasitaemia quantification (MP), the slides were stained with Giemsa stain and examined under the microscope using oil immersion objectives (WHO, 1991). Parasitaemia was quantified in thick films by counting parasites against white blood cells(Cheesbrough, 1999, Sowunmi, 1996).The intensity of parasitaemia was measured per high power field or microscopic field. Up to 5-10 high power fields were examined before intensity was confirmed and the number of parasites per field noted. Severity of parasitaemia was assessed as parasitic index (P1) and expressed as scanty, mild and severe (+, ++ and +++) (Melita *et al.*; 2001)..

2.2.1.1 Determination of Blood Cell Counts and Packed Cell Volume (PCV).

Total white blood cell count.

Principle: This is done with whole blood in which red blood cells have been lysed. The lytic agent is required to destroy the red blood cells and reduce red cell stroma to a residue that causes no detectable response in the counting system without affecting leucocytes in such a manner that the ability of the system to count them is altered. The diluent contains a reagent to lyze the red cells and another to convert haemoglobin to haemoglobin cyanide. The white cells area are counted by impedence or light scattering technology or both.

Normal ranges:

Children----------6.0--18.0 $x10^9$/L

Adult ----------5.0--8.0$x10^9$/L

Procedure: 0.02 ml of anticoagulated blood and 0.38ml turk's solution (a diluent) were pipetted into a test tube and mixed properly. Then 0.02 ml of the mixed solution was then pipetted into an already charged Newbauer machine and allowed to stand for 2-5 minutes. The set up was examined microscopically using x 10 light microscope.

Differential count: This differentiates the white blood cells into their different types/components.

Normal ranges:

Neutrophil----------40-80%

Lymphocyte----------20-40%

Monocyte----------2-10%

Eosinophil----------1-6%

Basophil----------1-2%

Procedure: Thin film of the blood specimen was done and allowed to air-dry. The smear was Giemsa stained and viewed microscopically using x100 oil immersion (Cheesbrough, 1998).

Packed Cell Volume

This is used as a simple screening test for anaemia and as a rough guide to the accuracy of haemoglobin measurements. It is done using Haematocrit reader method. The haematocrit x100 is about three times the haemoglobin expressed in g/l. In conjunction with estimations of haemoglobin and red blood cell count(RBC), it can be used in the calculation of red cell indices.

Normal ranges

Adult men----------36-45%

Adult women----------33-38%

Children----------27-33%

Procedure: A special capillary tube was filled with well mixed anticoagulated blood up to 2/3 of its length. The end of the tube was then sealed with plasticine. The filled capillary tube was place in the grooves of the haematocrit centrifuge head with the sealed end placed away from the centre of the centrifuge. the centrifuge was covered by screwing up the lid adequately and spun for five (5) minutes at 12,000 rpm. The haematocrit tubes were removed as soon as the centrifuge stopped spinning, placed on the haematocrit reader and read. The PCV of each individual was then calculated in percentage thus

$$PCV(\%) = \frac{\text{Length of red cell column (mm)}}{\text{Length of total column (mm)}} \times 100$$

2.2.2 Determination of Biochemical Parameters

2.2.2.1 Determination of Serum or Plasma Urea

Urea levels were determined using the diactetylmonozine methods (Kaplan, 1965). Urea is the main waste product of protein breakdown. It is formed in the liver by the reactions of the Krebs urea cycle, passed into the circulation and is excreted by the kidneys. However, not all the urea filtered by the glomeruli is excreted in the urine. Depending on the hydration state of a person, about 40-70% of the urea is passively reabsorbed with water and returned to the blood; the rate of absorption being inversely related to the rate of urine flow.

Principle: Urine reacts with diacetylmonoxine at high temperature in an acidic medium in the presence of cadmium ions and thiosmicarbazide. The absorbance of the red colour produced is then measured in a colorimeter using green filter 520nm (Ilford No. 604) or in a spectrophometer at 530nm wavelength.

Diacetylmonoxine urea method is calibrated from solution of urea which is prepared and diluted to make a series of standards. Urea test kits using the diacetylmonoxime method are available. Approximate urea reference (normal) ranges are:

Adults: 3.3 - 7.7mmol/l

Infants: 1.3 – 5.8mmol/l

Method: Heparin container was used for the collection of the sample and three test tubes were used in the analysis. Urea has three working reagents; the urease reagent (buffer and urease enzyme), phenol and hypochlorite. One hundred mililitres of reagent one (urease) was added to the three test tubes, 10mls of standard was added to the standard test tube while 10mls of the sample was added to the sample test tube. These were incubated for 10mins at 37^0c. Then 2.5mls each of the second and third reagents (phenol and hypochlorite) were added to each test

tube, mixed and re-incubated for another 20mins. The value was then read with spectrophotometer at 546nm.

Result = Absorbance of sample test X Concentration of standard

Absorbance of standard

2.2.2.2 Determination of Serum or Plasma Creatinine

Creatinine is a nitrogenous waste product formed from the metabolism of creatinine in skeletal muscles. It diffuses freely throughout the body water and is filtered from the extracellular fluid by the kidneys and excreted in the urine. The excretion of creatinine is relatively constant in the absence of disease. It is an important test of kidney function. It is less affected by factors such as age, dehydration, catabolic states and changes in diet than urea levels.

Principle: The Jaffe-Slot modified alkaline picrate colorimetric method was used. Creatinine level was determined by using a Hitachi 407 auto-analyser (Slot 1965, Thomas,1998). It is a kinetic method because the absorbance is taken stepwise until the end point is reached. This means that readings are taken at different points as the reaction progresses. This reduces interference from non-creatinine substances making the test more specific. It is calibrated from a solution of creatinine, prepared and diluted to make a series of standards. Creatinine reacts with picric acid in an alkaline medium and the absorbance of the yellow-red colour produced is measured in a colorimeter using a blue – green filter 490nm (Ilford No. 603). However, a number of other compounds similarly react with picric acid and may give artificially high result for creatinine. To avoid this, two readings are taken. The colour produced by creatinine is quickly destroyed by acid while that given by non-creatinine chromogens is destroyed slowly. To obtain the colour produced by true creatinine, the second reading which is due to non-creatinine substances is

subtracted from the first reading which is due to creatinine and non-creatinine substances.

Method: Two reagents were involved which are (1) sodium hydroxide and sodium bicarbonate and (2) picric acid. Five hundred mililitres of reagent (1) and same amount of reagent (2) were added into a blank test tube and used for zeroing the machine. The same value of these reagents was added to reagent standard tube and mounted into the machine and read immediately.

After 30 seconds, first reading, Absorbance (A) was taken. The second Absorbance (B) was taken after one hour and the final Absorbance(C) was obtained by subtracting Absorbance B from Absorbance A i.e. A-B. This same method was repeated for sample test.

Result = $\frac{\text{Absorbance of sample Test}}{\text{Absorbance of standard}} \times \text{Concentration of standard}$

2.2.2.3 Measurement of Serum Albumin

Principle: Albumin is produced in the liver and constitutes about 60% of total serum protein. It regulates the flow of water between the plasma and tissue fluid. Reduction in the concentration of albumin leads to oedema because the plasma osmotic pressure becomes insufficient to draw water from the tissue spaces back into the plasma. Another important function of the albumin is binding and activating substances. Reduction in its level leads to development of toxic effects due to increase in unbound substances. It diffuses through damaged membranes and is filtered by the kidneys. Any defect of the kidneys may affect the level or concentration of albumin.

Principle: The Bromo-cresol Green (BCG) binding method was used (Spencer and Price, 1977). The indicator is yellow between pH 3.5 – 4.2 but when it binds to albumin the colour of the indicator changes from yellow to blue-green. It is calibrated from a 50ggl solution of albumin. The absorbance of the colour produced is measured in a colorimeter using a spectrophotometer at 680nm wavelength.

Methods: Three mililitres of blood was spun with the centrifuge to separate the plasma from the cells. One mililitre of biuret reagent was added to each of both the sample test and standard test tubes. Then 10 microlitres of the sample test was added to the sample test tube while standard reagent was added to the standard test tube. These were mixed thoroughly and allowed to stand on the bench for 10mins. The value was then read with the spectrophotometer at wavelength of 680nm

Result: $\frac{\text{Absorbance of test sample}}{\text{Absorbance of standard}}$ X Concentration of the standard

2.2.3 Determination of Serum Electrolytes

3.2.3.1: Measurement of Serum or Plasma Sodium and Potassium

Serum electrolytes were investigated using Flame Emission Photometry Method (Overman and Davis, 1947). The proper balance of these electrolytes is critical for the normal functioning of the muscles, heart, and nerves. Sodium and potassium exist in the body as free ions and maintain cellular tonicity and fluid balance between the various cellular components. They are involved in metabolic processes, maintenance of pH and regulation of neural and muscular function. Measurement of sodium and potassium is usually done in the assessment of renal function.

Sodium – This is the main extracelluar cation. An increase in plasma sodium results in three compensatory mechanisms such as:

- thirst which prompts oral fluid intake
- anti-diuretic hormone (ADH) secretion from the pituitary is increased, leading to renal water retention
- a shift of water from intracellular to extracellular spaces.

Regulation of retained body sodium is maintained by the kidneys and excess excreted in the urine.

Potassium: This is the main intracellular cation. Ninety-eight percent of it is maintained within the cells by an ATP dependent mechanism known as sodium pump. In this mechanism, any sodium that diffuses into the cell is excreted in exchange for potassium. Insulin also enhances the cellular uptake of potassium. It plays important role in intracellular osmolarity, enzymatic reactions, regulation of muscles and transmission of nerve impulses. An important factor in the control of potassium is the acid/base status.

Method: Flame emission spectrometry method was used to measure the level of plasma sodium and potassium using a flame emission spectrometer (Cheesbrough 2005). It measures the concentration of these electrolytes in mmol/l.

Blood specimen was collected correctly and the serum separated from the cells within 2½ hours. A suitable anticoagulant, lithium heparin was used. The plasma or serum was diluted correctly using model 805 clinical Dilutor. Using compressed air, the diluted serum was sprayed as a fine mist of droplets into a non-luminous gas flame. This became coloured by the characteristic emission of the sodium or potassium metallic ions in the sample. Light of a wave length corresponding to the metal being measured was selected by a light filter and allowed to fall on a photosensitive detector system. The amount of light emitted depended on the

concentration of metallic ions present in the sample. The amount of electrolyte in the sample was therefore measured by comparing the amount of light emitted from the sample with that from a standard solution.

2.2.3.2: **Measurement of Serum Bicarbonate and Chloride ions**

Bicarbonate: This is the second largest fraction of the anions in plasma. The serum bicarbonate comprises of 95% of the total carbondioxide content; thus the measure of total CO_2 can be used as an excellent estimator of serum bicarbonate. The kidneys and lungs maintain daily acid-base balance. Bicarbonate content of serum is therefore a significant indicator of electrolyte dispersion and anion deficit. It often provides important data used in the care of critically ill patients. Together with pH determination, its measurements are used in the diagnosis of serious disorders associated with acid-base imbalance. Some of these conditions are hyperkalemic acidosis, renal failure, renal tubular acidosis and ketoacidiosis (Narins and Gardener, 1981).

Reference Values:

Males

12-24 months: 17-25mmol/L

3 years: 18-26mmol/L

4-5 years: 19-27mmol/L

6-7 years: 20-28mmol/L

8-17 years: 21-29mmol/L

> or =10 years: 22-29mmol/L

Females:

1-3 years: 18-25mmol/L

4-5 years: 19-26mmol/L

6-7 years: 20-27mmol/L

8-9 years: 21-28mmol/L

> or = 10 years: 22-29mmol/L

Technique: An autoanalyzer was used for measuring total CO_2 content. This method measures amount of CO_2 liberated from the sample after addition of strong acid. The CO_2 produced in this reaction diffuses across a dialysis membrane. A bicarbonate -carbonate buffer solution containing an indicator dye absorbs the CO_2 and a colorimeter then evaluates the new colour, which it converts to total CO_2.

Chloride ions: Chloride is an inorganic anionic halogen with an atomic weight of 35.5. It is distributed exclusively within the extracellular fluid compartment which comprises the blood/plasma compartment and the interstitial fluid compartment. It is the major anion associated with sodium in the extracellular fluid compartment. The kidneys are responsible for the maintenance of total body chloride balance. They maintain homeostasis because each kidney is composed of functional units, nephrons. Part or all of the chloride filtered by the initial portion of each nephron, the glomerulus, is reabsorbed as a result of both active and passive transport processes along the tubules comprising each nephron (Kokko and Jacobson, 1985). An increased level of blood chloride known as hyperchloremia usually indicates dehydration but can also occur with other problems such as Cushing syndrome or kidney disease. A decreased level of blood chloride known as hypochloremia occurs with any disorder that causes low blood sodium.

Reference values:

Adults: 98-106mmol/L

Premature neonates: 95-110mmol/L

Neonates, cord blood: 96-104mmol/L

Neonates, 0 to days: 98-113mmol/L

Children, older than 30 days: 98-107mmol/L (Moe and Fuster, 2003).

Technique: There are several techniques for determination of chloride. In this study, the autoanalyzer method was used (Smith *et al*.,1999). Here, the chloride ions displace thiocyanate from mercuric thiocyanate. The free thiocyanate then reacts with ferric ions to form a coloured complex, ferric thiocyanate, which is measured photometrically.

2.2.4 Investigation of Urine Samples.

Each subject completely emptied his or her bladder and the voided urine was tested for protein. The density/specific gravity of the urine samples was also determined using Urinometer method (Cheesbrough,2005).

Testing urine for protein – When more than trace amounts of protein is found in urine, the condition is referred to as proteinuria – or albuminuria. It occurs when there is glomerular damage.

Protein reagent strip method – This detects mainly albumin. The test area is impregnated with tetrabromophenol blue or tetrabromophenolphthalein ethylester indicator and buffered to an acid pH (Cheesbrough 2005). If protein is present in

urine, the indicator changes colour from light yellow to green-blue depending on the amount of protein present. It is sensitive detecting as little as 0.1g/l of albumin. Albustix which is commercially available protein test strip was used. The scale ranges from negative, trace, +0.3g/l, ++ 1g/l, +++3g/l and ++++ 20g/l.

2.2.4.1 Measuring urine density (specific gravity)

This is a measure of the density of the substances dissolved in the urine and it depends on the number of dissolved particles and their masses. It provides information on the concentrating and diluting ability of the kidneys.

Urinometer method: This technique uses specially calibrated hydrometer. It is calibrated from 1.000 – 1.060 with each division being equal to 0.001. The principle is, the lower the concentration of solutes in the urine, the further the urinometer will sink in the urine. The accuracy of the urinometer is checked by floating it in distilled water. The reading is 1.000 at the specified temperature on the urinometer. If the reading differs from 1.000, the difference is subtracted from each urine reading.

Procedure: At least 50ml of urine sample of each subject was obtained and transferred to a detergent free cylinder. The urinometer was immersed in the urine making sure that it floated centrally and did not touch the bottom or sides of the cylinder. The reading was then taken from the lowest point of the meniscus at eye level (Cheesbrough, 1992).

Interpretation: The normal range for urine relative mass density is 1.010 – 1.030. Therefore a consistent low urine mass density usually indicates poor tubular re-absorption. Excessive fluid intake also results to low urine pH. In general, the greater the volume of urine output, the lower is its pH and the lighter its colour.

A high value of urine pH may be as a result of the presence of such substances not normally found in urine such as protein or glucose. Heavy perspiration and dehydration may also result to high urine pH. The scale ranges from Negative, 5.5, 14, 28, 55 to 111mmol/L.

2.2.4.2 Measurement of Urine pH:

Urine pH is used to classify urine as either a dilute acid or base solution. The glomerular filterate of blood is usually acidified by the kidneys from a pH of approximately 7.4 to a pH of about 6 in the urine. The lungs and kidneys are the main regulators of an organism's acid/alkali balance. The kidneys maintain normal acid-base balance primarily through the re-absorption of sodium and the tubular secretion of hydrogen and ammonium ions. The pH of urine can vary between 4.5 and 8 with the first urine produced in the morning generally being more acidic and the urine produced after meals generally more alkaline. Normal reference values are not provided for urine pH as the variation is too wide and results have to be considered in the context of the other quantifiable parameters. Urine becomes increasingly acidic as the amount of sodium and excess acid retained by the body increases. Secretion of an acid or alkaline urine by the kidneys is one of the most important mechanisms the body uses to maintain a constant body pH. Changes in the pH of the urine can signal that a patient is suffering from some underlying disease (Narins and Emmett, 1980).

Steps in the in the measurement of pH: The pH paper is lined with chemicals which react with acids and bases and change/ colour based on the pH of the solution used. The pH paper was dipped into the urine samples. The colour of the paper which was been exposed to the urine was immediately compared to the chart which is attached to the roll of pH paper. The colour that the pH paper turned immediately after being dipped into the sample liquids corresponded to the pH of the urine.

Numbers higher or lower than the normal values indicated problems with acids or bases within the body.

2.3 Statistical Analysis

Data were entered into a computer and analysed using SPSS version 20.0 for windows (SPSS Inc. Chicago, IL: USA). Differences and proportions were tested by Chi-square tests and student's t tests for trend or independence as appropriate. Multiple logistic regression analysis was used to show whether there was significant correlation between malaria infection and impairment of renal function. A P value of < 0.05 was taken as significant.

CHAPTER THREE

3.0 RESULTS

3.1 Characteristics of Study Population

A total of 240 individuals were investigated in this study. Out of the above number, 90(37.50%) were positive for malaria parasite while 150(62.50%) were negative. Of the 240 individuals examined, 107(44.58%) were males while 133(55.42%) were females. The mean age ± standard deviation is 30.19±14.69 years. Probability ≤ 0.05 shows significant difference while $P > 0.05$ shows no significant difference.

3.2 Prevalence of Malaria Parasitaemia

The overall prevalent rate of parasitaemia in the study population is 37.50% (n = 90). The prevalence due to sex shows that equal number of males and females were positive for malaria parasite (Table 2). In the prevalence due to age, the age range 10 – 16 years had the highest prevalence 14(56.0%) followed by the age range 24-30 years 27(48.2%) while no individual was positive at the range 66 – 72 years (Table 2). However, the differences between the age groups were not statistically significant ($P>0.05$).

3.2.1 Categories of MP Prevalence among Positive Individuals.

Among the 90 malaria positive individuals, 70 (77.8%) had mild infection, 13(14.4%) had moderate infection while 7(7.8%) had severe infection (Table 3). According to sex, 35(50.0%), 8(61.5%) and 2(28.6%) males had mild, moderate and severe infection respectively. On the other hand, 35(50.0%), 5(38.5%) and 5(71.4%) females had mild, moderate and severe infection respectively (Table 3).

Among the age groups, 52 – 58 years, had only mild infection 4(100.0%), moderate infection was highest in 38 – 44 years age group while the rate of severe infection was highest in the 3 – 9 years age group 1(50.0%).

3.2.2 Mean Parasite Density of Malaria Positive Individuals

The mean parasite density of the 90 positive individuals is 2361.89±857.55p/µ. That of positive males is 1854±1066.59p/µ while the positive females had 2869.11±1351.19p/µ (Table 4). The mean parasite density is highest in 45 – 51 years age group (7876.67±7307.73p/µ) and least in the age group 52 – 58 years (165.00±26.30p/µ) (Table 4).

3.2.2.1 Mean Parasite Density in Different Categories of Infection

The mean parasite density of the malaria parasite is highest in severe infection, followed by the moderate and least in mild infections (Table 5).

3.3 BIOCHEMICAL PARAMETERS OF MALARIA POSITIVE AND NEGATIVE INDIVIDUALS

The levels of total protein, urea, albumin and creatinine in malaria positive individuals are higher than those of their negative counterparts. However, the difference in the levels of albumin of positive and negative individuals is not statistically significant ($P>0.05$) (Table 6). Correlation analysis showed that there is positive but insignificant association between malaria parasitaemia and total protein and albumin ($r= 0.198$ and $r= 0.052$) respectively(Figures 4 and 7). Significant positive association, however, exists between MP, urea and creatinine ($r= 0.348$ and $r= 0.602$) (Figures 5 and 6).

Table 2: Sex and Age Prevalence of Malaria Positive Individuals

	Number examined	n (%)
Overall	240	90(37.50)
Sex		
Male	107	45(42.1)
Female	133	45(33.8)
X^2	1.710	
Significance or P value	0.228^{ns}	
Age(years)		
3-9	13	2(15.4
10-16	25	14(56.0)
17-23	49	18(36.7)
24-30	56	27(48.2)
31-37	34	12(35.3)
38-44	20	4(20.0)
45-51	18	6(33.3)
52-58	13	4(30.8)
59-65	7	3(42.9)
66-72	5	0(0)

x^2 = 15.27

P value = 0.084

Table 3: Overall, Age and Sex Categories of MP Prevalence

	N	MILD n(%)	Moderate n(%)	Severe
Overall	90	70(77.8)	13(4.4)	7(7.8)
Sex				
Male	45	35(50.0)	8(61.5)	2(28.6)
Female	45	35(50.0)	5(38.5)	5(71.4)
Age(years)				
3-9	2	1(50.0)	0(0)	1(50.0)
10-16	14	10(71.4)	2(14.3)	2(14.3)
17-23	18	15(83.3)	2(11.1)	1(5.6)
24-30	27	25(92.6)	1(3.7)	1(3.7)
31-37	12	8(66.7)	4(33.3)	0(0)
38-44	4	1(25.0)	2(50.0)	1(25.0)
45-51	6	4(66.7)	1(16.7)	1(16.7)
52-58	4	4(100.0)	0(0)	0(0)
59-65	3	2(66.7)	1(33.3)	0(0)

Table 4: Overall, Sex and Age Mean Parasite Density of Malaria Individuals

	Number positive	Mean ± S.E(p/µ)
Overall	90	2361.89±857.55
Male	45	1854.671066.59
Female	45	2869.11±1351.19
P value		0.557^{ns}
Age(years)		
3-9	2	$5320.0±5080.00^{ab}$
10-16	14	$6074.27±4118.971^{a}$
17-23	18	$1567.78±1116.024^{ab}$
24-30	27	$324.07±63.79^{b}$
31-37	12	$525.00±160.89^{ab}$
38-44	4	$5795.00±3272.92^{ab}$
45-51	6	$7876.67±7307.73^{a}$
52-58	4	$165.00±26.30^{ab}$
59-65	3	$840.00±680.00^{ab}$

In age groups, mean parasite density with different alphabet show significant difference ($P<0.05$).

In sex, '^{ns}' = no significant difference.

Table 5: Comparison of mean Parasite density in different MP categories.

MP-Category	Number examined	Mean + S.E(p/µ)
Mild	70	$246.\ 43±11.56^{b}$

Moderate	13	2040.00±704.78[b]
Severe	7	2.41E4±7213.73[a]

Mean parasite densities with different alphabet show significant difference ($P<0.05$).

Table 6: Comparing Biochemical Parameters for Malaria Positive and negative Individuals.

Biochemical Parameters	Malaria positive (n = 90)	Malaria negative (n=150)	p value
Total protein(mg/dl)	75.54 ±14.30	71.0710.53	0.011*
Urea(mmol/L)	4.76 ± 2.76	4.08±1.69	0.018*
Albumin(mg/dl)	45.84±6.38	44.72±5.16	0.161^{ns}
Creatinine(µmol/L)	92.54±27.09	76.26±19.31	0.0001**

** Significant difference (P<0.05)

* Significant difference (P<0.05)

^{ns} no significant difference (P>0.05)

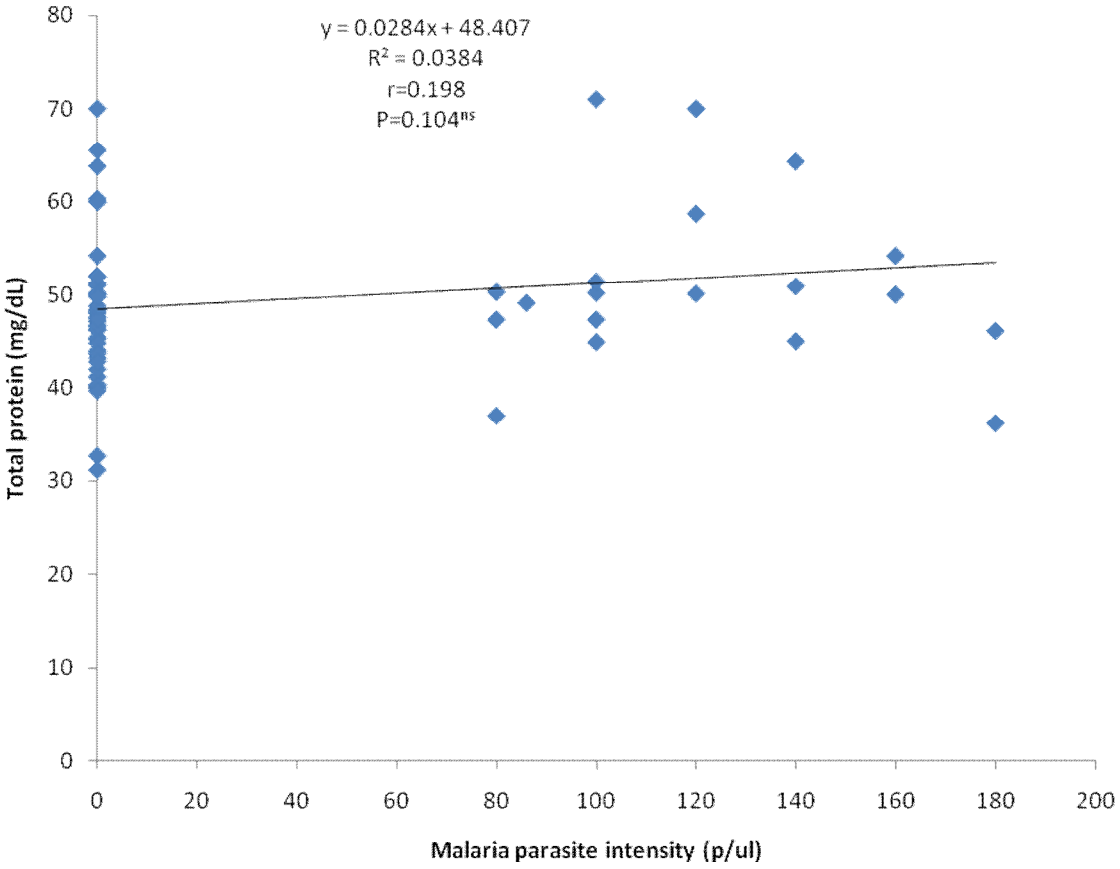

Fig 4: Malaria Parasite Intensity in relation to Total Protein

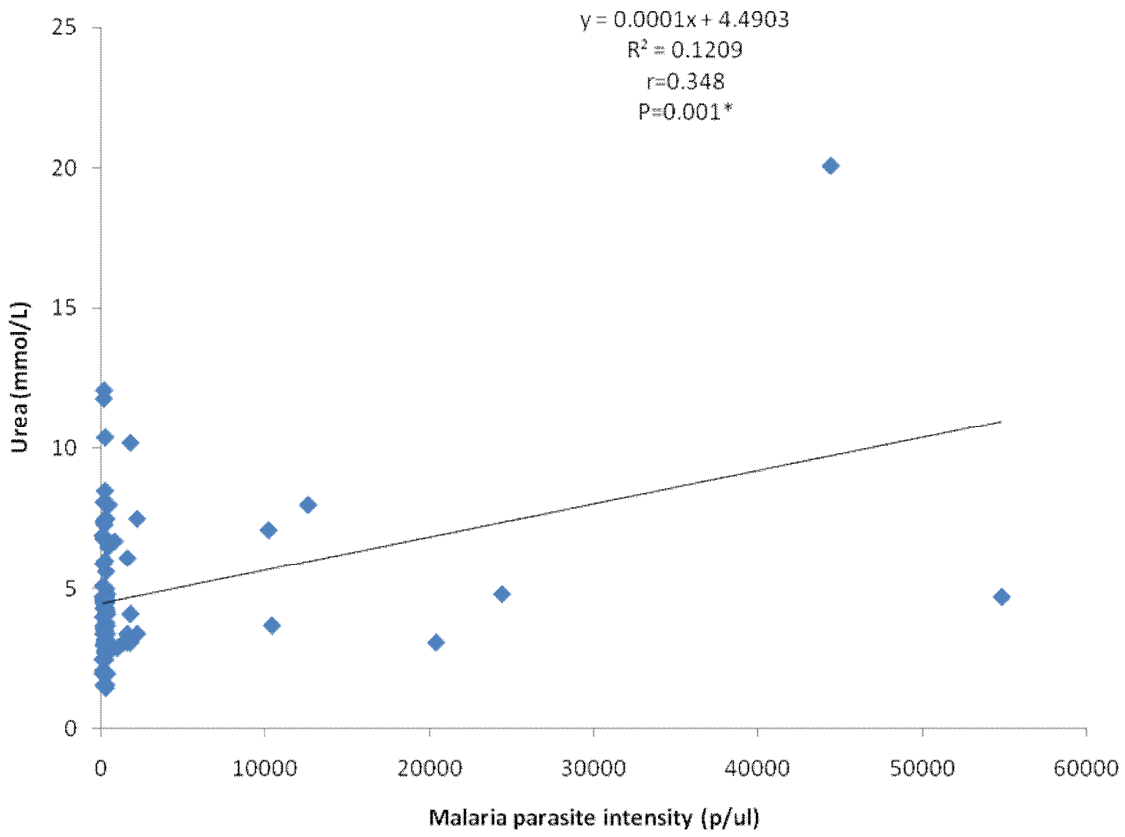

Fig 5: Malaria Parasite Intensity in relation to Urea

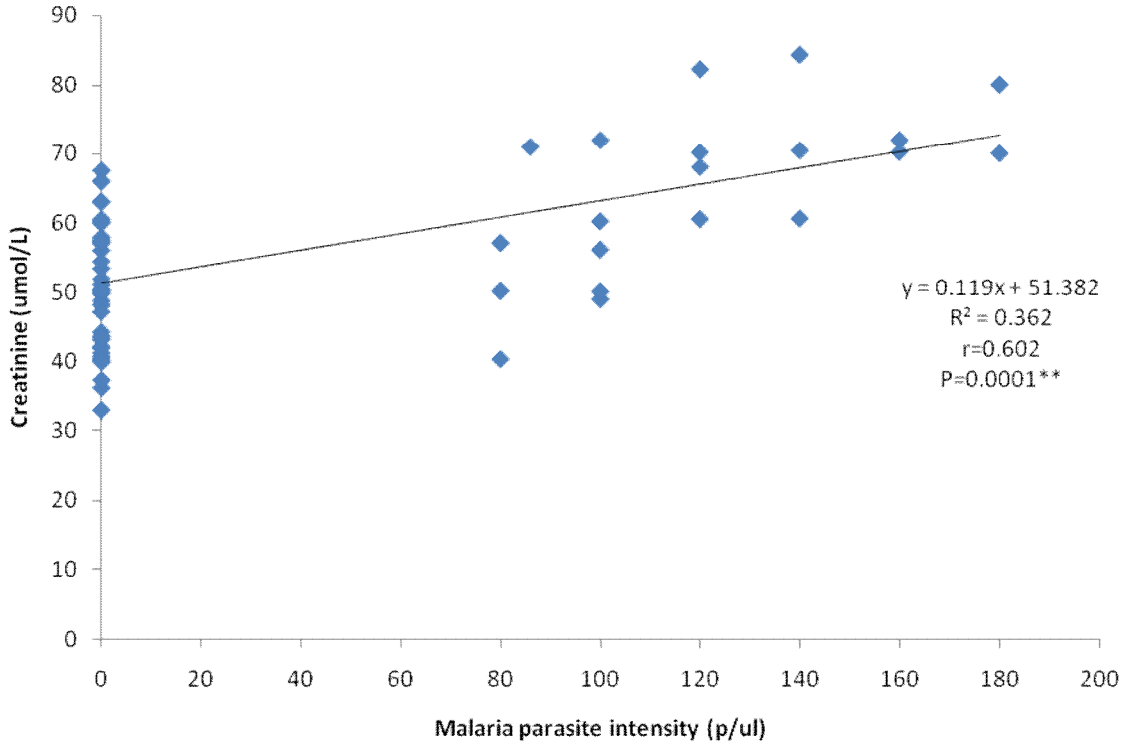

Fig. 6: Malaria Parasite Intensity in relation to Creatinine

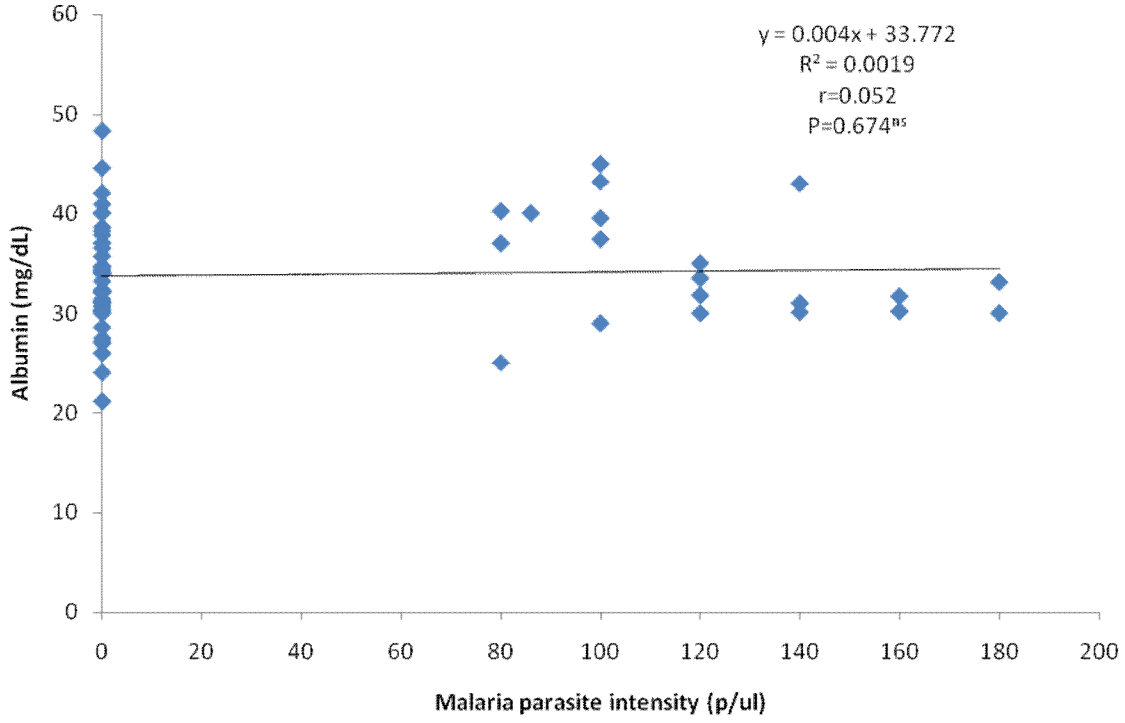

Fig. 7: Malaria Parasite Intensity in relation to Albumin

3.3.1: Biochemical Parameters of Malaria Positive and Control Individuals due to Sex.

The mean values of total protein, urea, albumin and creatinine are higher in malaria positive males than their negative counterparts but the differences are significant only in the values of total protein ($P < 0.05$) and creatinine ($P < 0.01$) (Table 7). In the same way, the mean values of total protein, urea, albumin and creatinine are higher in positive females than in the negative females but the difference is only significant in the creatinine value ($P < 0.05$) (Table 7).

3.3.2: Age Comparison of Biochemical Parameters of Malaria Positive and Negative Individuals.

The mean values of total protein and urea are higher in positive individuals of all age classes except in the age class 3 – 9 years where the values of both parameters are higher in the negative individuals(Table 8).

The difference in the values of total protein is significant only in the age class 52 – 58 years ($P < 0.01$) but the differences in the mean values of urea are not significant ($P > 0.05$). The mean values of Albumin of positive individuals are higher in all the age classes except in the classes 31 – 37 years and 38 – 44 years. In all the age classes, the differences are not significant ($P > 0.05$) (Table 8). The mean values of serum creatinine of all the age classes are higher in positive individuals than in the controls but the differences are significant only in the 17 – 23 years and 38 – 44 years age classes ($P < 0.05$) (Table 8).

Table 7: Sex Comparison of Biochemical Parameters of Malaria Positive and Negative Individuals.

Sex	Biochemical Parameter	Malaria Positives (n = 45)	Malaria Negatives (n = 62)	P value
Males	TP(mg/dl)	75.61±11.21	70.81±10.38	0.026*
	Urea(mmol/L)	4.99±3.29	4.14±1.35	0.067ns
	Albumin(mg/dl)	44.76±6.87	44.37±4.12	0.717ns
	Creatinine(µmol/L)	102.21±30.14	79.72±25.51	0.0001**
Females	TP(mg/dl)	75.46±16.99	71.25±10.68	0.135ns
	Urea(mmol/L)	4.54±2.12	4.05±1.90	0.919ns
	Albumin(mg/dl)	4.69±5.73	4.96±5.79	0.068ns
	Creatinine(µmol/L)	82.87±19.63	73.82±18.14	0.012*

** = Significant difference (P<0.01)

* = Significant difference (P<0.05)

ns = no significant difference (P>0.05)

3.4: **Serum Electrolytes of Malaria Positive and Negative Individuals**

The serum electrolytes of malaria positive and negative individuals showed no significant differences. The mean specific gravity of the positive individuals is slightly higher than those of their negative counterparts but the difference is not significant (P>0.05) (Table 9). Malaria parasitaemia positively correlated with the serum electrolytes but the association was not significant except with the bicarbonate ions (r= 0.278) (Figures 8-13).

3.4.1: Sex Comparison of Serum electrolytes of Malaria Positive and Negative Individuals.

The mean serum values of sodium ions, bicarbonate ions, specific gravity and pH are higher in positive males than in negative males while the mean values of serum potassium and chloride ions are slightly higher in negative males than in positive males. The differences, however, are not significant (P > 0.05) (Table 10).

In females, the mean serum levels of sodium ions, chloride ions and bicarbonate ions are slightly higher in the negative individuals. The level of potassium ion is higher in the positive females while specific gravity and pH are the same in the both positive and negative females(Table 10).

Table 8: Age Comparison of Biochemical Parameters of Malaria Positive and Control Individuals.

Age (yrs)	TP(mg/dl)			UREA(mmol/L)			AIB(mg/dl)			CREAT(µmol/L)		
	Pos	Neg	P value	Pos	Neg	P value	Pos	Neg	P value	Pos	Neg	P value
3 – 9	70.25±13.78	73.58±7.71	0.719^{ns}	3.7±0.00	4.30±1.77	0.289^{ns}	44.10±0.14	42.57±3.84	0.218^{ns}	80.25±8.13	75.88±24.16	0.656^{ns}
10 – 16	70.85±10.23	70.55±10.83	0.941^{ns}	3.81±1.17	3.23±0.80	0.157^{ns}	46.26±6.21	43.84±3.96	0.249^{ns}	90.16±33.11	74.57±20.42	0.162^{ns}
17 – 23	79.85±24.54	71.14±10.69	0.168^{ns}	4.54±2.79	3.47±1.09	0.063^{ns}	45.51±7.21	44.35±5.24	0.558^{ns}	90.59±29.32	72.80±18.26	0.029*
24 – 30	73.10±8.62	71.97±10.70	0.605	4.56±2.39	4.12±1.56	0.421^{ns}	46.17±5.93	44.93±5.33	0.417^{ns}	89.32±22.07	79.46±17.88	0.074^{ns}
31 – 37	74.35±8.59	70.87±11.43	0.325^{ns}	5.04±2.75	5.05±2.07	0.995^{ns}	45.07±6.47	46.25±4.55	0.586^{ns}	89.22±25.34	71.12±24.91	0.057^{ns}
38 – 44	74.65±10.00	72.34±10.10	0.689^{ns}	5.72±2.23	4.52±2.61	0.392^{ns}	45.35±10.12	46.61±6.73	0.825^{ns}	107.30±21.84	71.22±15.57	0.038*
45 – 51	75.53±14.41	67.90±13.16	0.304^{ns}	7.5±6.22	3.83±1.54	0.055^{ns}	45.10±4.17	44.61±4.67	0.825^{ns}	100.23±27.64	80.83±12.54	0.152^{ns}
52 – 58	88.48±5.65	65.76±8.69	0.001*	5.25±1.53	3.86±0.71	0.168^{ns}	44.60±2.25	44.39±4.29	0.910^{ns}	88.73±23.57	87.74±12.50	0.941^{ns}
59 - 65	88.27±14.30	76.52±7.43	0.291^{ns}	4.70±2.44	3.73±0.67	0.564^{ns}	50.8±13.27	43.15±4.09	0.424^{ns}	135.77±31.69	77.88±21.42	0.064^{ns}

* = significant difference ($p<0.05$)

ns = no significant difference ($p>0.05$)

Table 9: Serum electrolytes of Malaria Negative and Positive Individuals.

Serum electrolytes	MP (n= 90)	(n=150) (Non-MP)	P value
Sodium ion(mmol/L)	$1.7x10^2$±3.38	$1.37x10^2$±3.76	0.992^{ns}
Potassium ion(mmol/L)	3.69±0.42	3.77±0.59	0.264^{ns}
Chloride ion(mmol/L)	$1.06x10^2$±3.44	1.05±3.75	0.374^{ns}
Bicarbonate ion(mmol/L)	22.78±3.38	23.14±3.73	0.459^{ns}
Specific Gravity	21.43±11.71	1.02±0.01	0.083^{ns}
PH	6.21±o.96	6.26±0.96	0.692^{ns}

ns = no significant different (p>o.05).

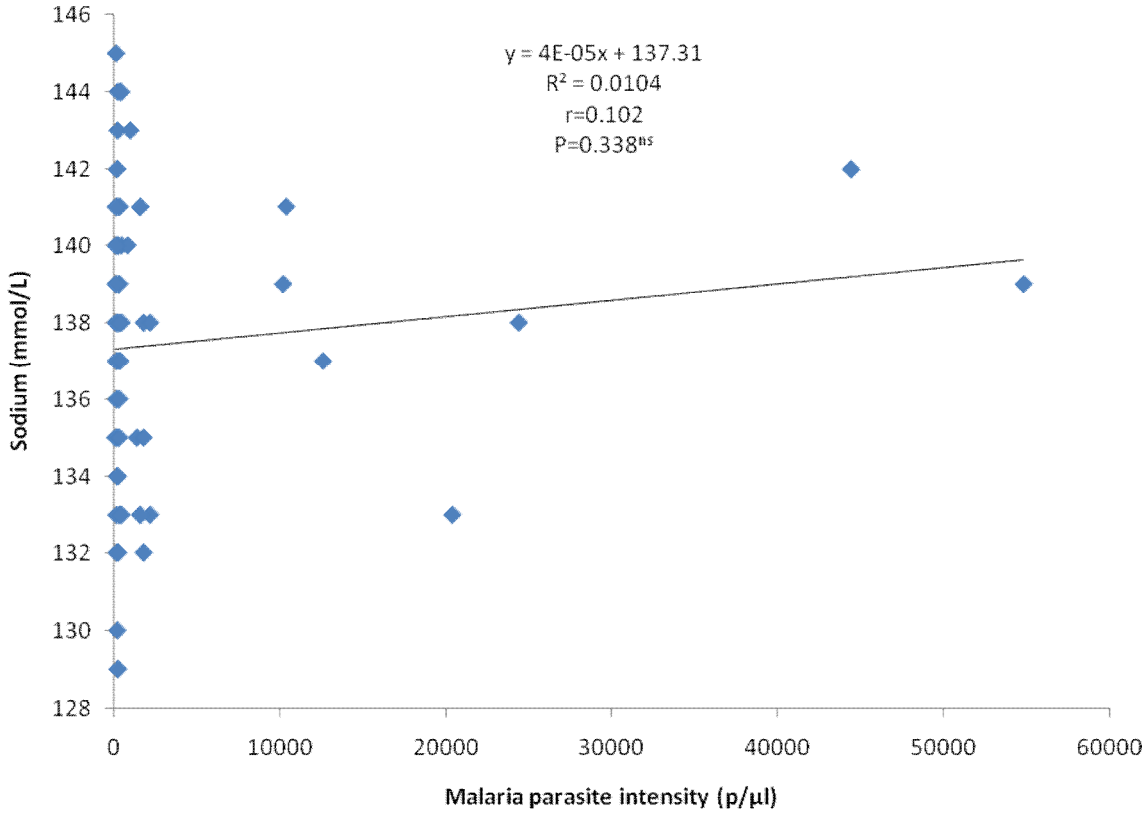

Fig. 8: Malaria Parasite Intensity in relation to Sodium ions

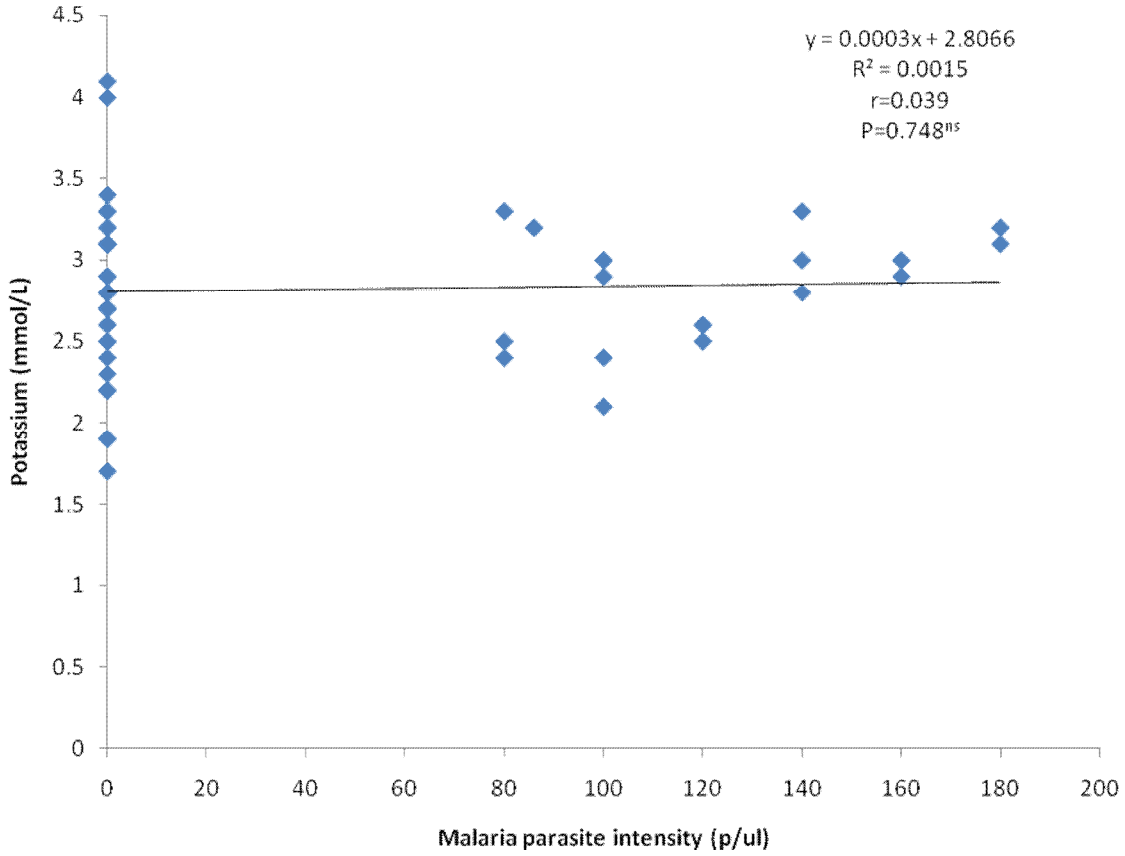

Fig. 9: Malaria Parasite Intensity in relation to Potassium Ions

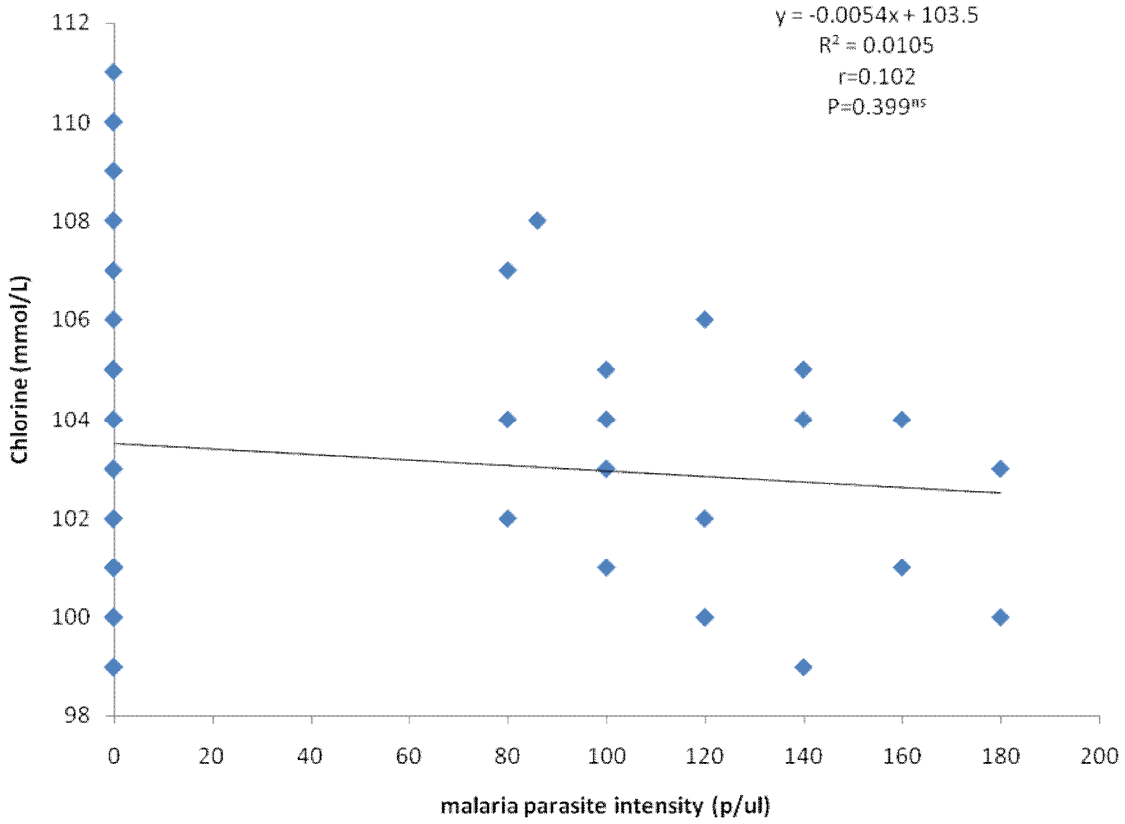

Fig. 10: Malaria Parasite Intensity in relation to Chloride Ions

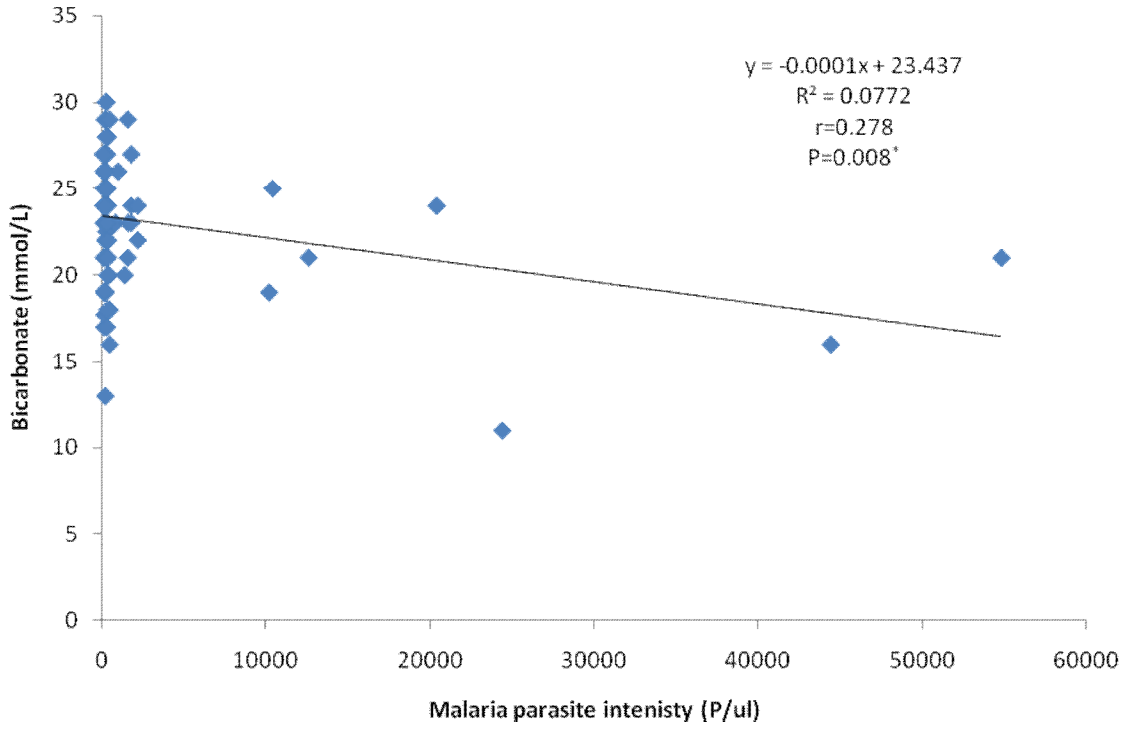

Fig. 11: Malaria Parasite Intensity in relation to Bicarbonate ions

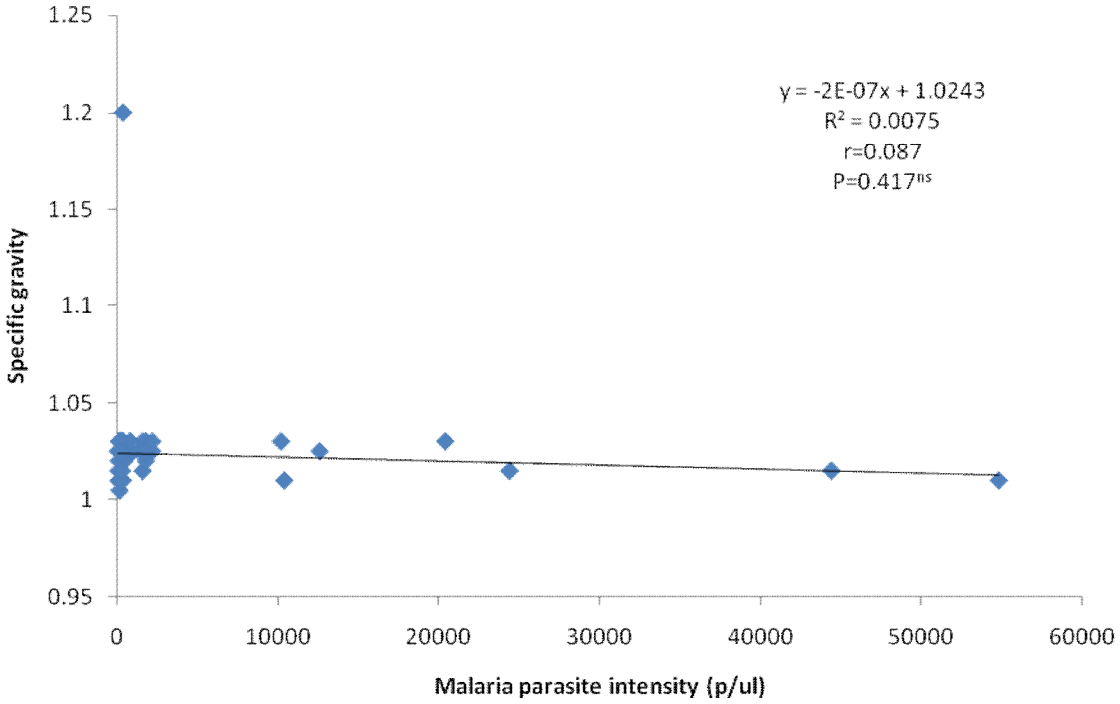

Fig. 12: Malaria Parasite Intensity in relation to Specific Gravity

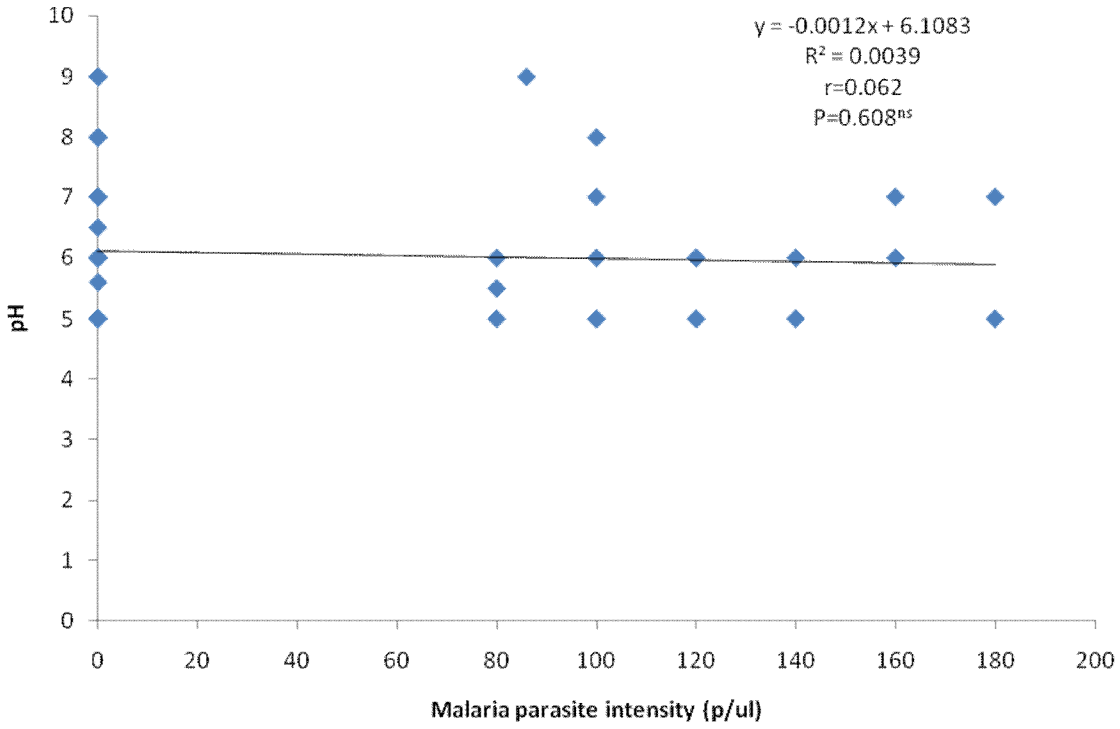

Fig. 13: Malaria Parasite Intensity in relation to pH

Table 10: Sex Comparison of Serum Electrolytes of Malaria Positive and Negative Individuals

Sex	Serum electrolyte	Positives (n = 45)	Negatives (n = 62)	P value
Males				
	Na^+(mmol/L)	137.80±3.29	136.79±4.33	0.174^{ns}
	K^+(mmol/L)	3.62±0.55	3.72±0.45	0.336^{ns}
	Cl^-(mmol/L)	105.84±3.79	106.29±3.54	0.538^{ns}
	HCO_3^-(mmol/L)	24.23±3.26	22.95±3.51	0.056^{ns}
	SG	1.03±0.03	1.02±0.01	0.248^{ns}
	pH	6.41±1.00	6.35±0.99	0.743^{ns}
Females	Na^+(mmol/L)	137.02±3.47	137.84±3.25	0.192^{ns}
	K^+(mmol/L)	3.91±0.62	3.68±0.411	0.023*
	Cl^-(mmol/L)	105.87±3.75	106.28±3.40	0.532^{ns}
	HCO_3^-(mmol/L)	22.05±3.88	22.66±3.30	0.369^{ns}
	SG	1.02±0.01	102±0.01	0.266^{ns}
	pH	6.11±0.92	6.11±0.92	0.988^{ns}

* = Significant difference between positives and negatives ($P<0.05$)

ns = No significant difference between positives and negatives ($P>0.05$)

3.4.2: Age Comparison of Serum electrolytes in Malaria Positive and Negative Individuals.

The mean values of serum sodium ions of malaria positive subjects were higher at age classes 3 – 9 years, 10 – 16 years, 17 – 23 years, 31 – 37 years, 38 – 44 years and 52 – 58 years than in the malaria negative individuals. In the age classes 24 – 30 years, 45 – 51 years and 59 – 65 years, the values were slightly higher in the malaria negative individuals. However, the differences were only significant in the age class 3 – 9 years ($P < 0.05$) (Table 11). The mean potassium values were higher in malaria positive individuals than their malaria negative counterparts in the age classes 17 – 23 years, 24 – 30 years, 31 – 37 years, 38 – 44 years, 52 – 58 years and 59 – 65 years. On the other hand, the values are higher in malaria negative individuals in the following age classes; 3 – 9 years, 10 – 16 years and 45 – 51 years. The differences were only significant in the age class 3 – 9 years. Chloride levels are higher in the control individuals in all the age classes except those of 17 – 23 years and 52 – 58 years where the malaria positive individuals had higher values. However, the differences were not statistically significant ($P > 0.05$) (Table 11). The mean bicarbonate levels were higher in malaria individuals in the following age classes; 3 – 9 years, 17 – 23 years, 24 – 30 years, 31 – 37 years and 52 – 58 years. In the age classes 10 – 16 years, 38 – 44 years, 45 – 51 years and 59 – 65 years, the values were higher in the control individuals but the differences were not significant ($P > 0.05$) (Table 11).

The mean values of specific gravity were the same in both infected and control individuals in all the age classes except in those of 17 – 23 years and 38 – 44 years where the infected individuals had higher values than the control. The mean pH values of infected individuals were higher than those of the controls in the age classes 3 – 9 years, 17 – 23 years, 24 – 30 years, 31 – 37 years, 52 – 58 years and 59 – 65 years while the controls had higher values in the 10 – 16 years, 38 – 44 years

and 45 – 51 years age classes. The difference is only significant in the 10 – 16 years age class ($P < 0.05$) (Table 11).

Table 11: Age Comparison of Electrolytes of Malaria Positive and Control Individuals.

Age(yrs)	Na^+(mmol/L)			K^+(mmol/L)			Cl^-(mmol/L)			HCO_3^- (mmol/L)			SG			pH		
	Pos	Neg	P value	Pos	Neg	P value	Pos	Neg	P value	Pos	Neg	P value	Pos	Neg	P value	Pos	Neg	P value
3 – 9	142.0±1.41	137.18±2.82	0.039*	3.25±0.07	4.08±0.83	0.008*	103.50±2.12	106.45±2.46	0.255^{ns}	26.00±1.41	23.81±2.96	0.201^{ns}	1.02±0.01	1.02±0.01	0.667^{ns}	6.50±0.71	6.18±1.17	0.651^{ns}
10 – 16	137.45±2.70	135.86±3.63	0.220^{ns}	3.57±0.78	3.64±0.39	0.789^{ns}	105.57±3.90	106.00±3.16	0.764^{ns}	21.79±4.58	22.64±4.11	0.633^{ns}	1.02±0.01	1.02±0.01	0.384^{ns}	5.68±0.67	6.59±0.89	0.011*
17 – 23	137.44±3.13	135.45±3.94	0.058^{ns}	3.74±0.40	3.61±0.24	0.155^{ns}	107.06±3.52	105.19±3.04	0.07^{ns}	23.03±3.89	22.42±3.08	0.574^{ns}	1.03±0.04	1.02±0.01	0.255^{ns}	6.50±0.97	6.16±0.84	0.225^{ns}
24 – 30	137.15±2.81	137.79±3.43	0.443^{ns}	3.90±0.53	3.77±0.29	0.273^{ns}	106.25±3.77	107.10±3.51	0.390^{ns}	23.51±3.80	22.72±3.60	0.433^{ns}	1.02±0.01	1.02±0.01	0.519^{ns}	6.50±1.08	6.38±1.11	0.682^{ns}
31 – 37	138.75±362	138.32±3.64	0.743^{ns}	3.84±0.40	3.67±0.53	0.295^{ns}	105.50±4.10	106.23±4.75	0.645^{ns}	24.17±2.86	23.00±4.16	0.343^{ns}	1.02±0.01	1.02±0.01	0.669^{ns}	6.33±1.07	6.18±0.82	0.676^{ns}
38 – 44	138.25±2.99	137.31±3.14	0.603^{ns}	3.88±0.21	3.76±0.38	0.417^{ns}	104.75±2.22	105.88±3.32	0.444^{ns}	21.75±2.99	22.25±3.45	0.783^{ns}	1.03±0.01	1.02±0.01	0.592^{ns}	6.00±0.82	6.09±1.13	0.856^{ns}
45 – 51	137.83±4.26	138.50±2.81	0.738^{ns}	3.37±0.50	3.60±0.32	0.332^{ns}	103.33±3.27	106.75±2.77	0.056^{ns}	21.83±3.66	23.17±3.27	0.469^{ns}	1.02±0.01	1.02±0.01	0.687^{ns}	5.75±0.88	6.29±0.86	0.244^{ns}
52 – 58	139.75±3.30	137.67±3.78	0.351^{ns}	3.85±0.46	3.47±0.45	0.218^{ns}	107.50±5.80	107.22±2.95	0.932^{ns}	26.00±2.16	23.00±2.55	0.066^{ns}	1.02±0.01	1.02±0.00	0.128^{ns}	6.25±0.29	5.67±0.66	0.125^{ns}
59 - 65	133.33±1.53	137.25±6.19	0.299^{ns}	4.27±1.85	3.73±0.31	0.578^{ns}	103.67±0.58	108.75±4.11	0.093^{ns}	21.33±2.52	22.75±3.86	0.583^{ns}	1.02±0.01	1.02±0.01	0.939^{ns}	6.33±1.16	5.75±0.96	0.517^{ns}

* = significant difference ($P<0.05$)

ns = no significant difference ($P>0.05$)

3.5: Haematological Parameters of Malaria Positive and Negative Individuals

The Packed cell volume, monocyte counts and the lymphocytes of positive individuals are lower than those of negative individuals while the

total leucocyte counts, neutrophil counts, basophils and eosinophil counts were higher in positive individuals than the negative ones. However, the differences were significant in the packed cell volume, total leucocyte counts, eosinophils and monocytes only (Table 12). Correlation analysis of malaria parasitaemia and haematological parameters showed positive but insignificant association except with the total leucocyte counts (r=0.563) (Figures 14-17).

Table 12: Haematological Parameters of Malaria Positive and Control Individuals

Haematological Parameters	MP (n = 90)	Non-MP (n =150)	P value
Packed cell volume(%)	39.41 ± 4.62	41.27±3.65	0.001*
Total leucocyte count ($x10^9$)/L/	5.86±1.38	5.31±0.66	0.001*
Neutrophils(%)	40.69 ± 9.98	38.15±10.53	0.061^{ns}
Lymphocytes(%)	52.81±12.98	53.41±9.03	0.676^{ns}
Eosinophils(%)	4.40±3.18	2.19±2.70	0.0001*
Monocytes(%)	3.32±1,84	4.12±2.46	0.008*
Basophils(%)	0.067±0.03	0.026±0.013	0.209^{ns}

* significant difference at $p < 0.05$

ns = no significant difference (P>0.05)

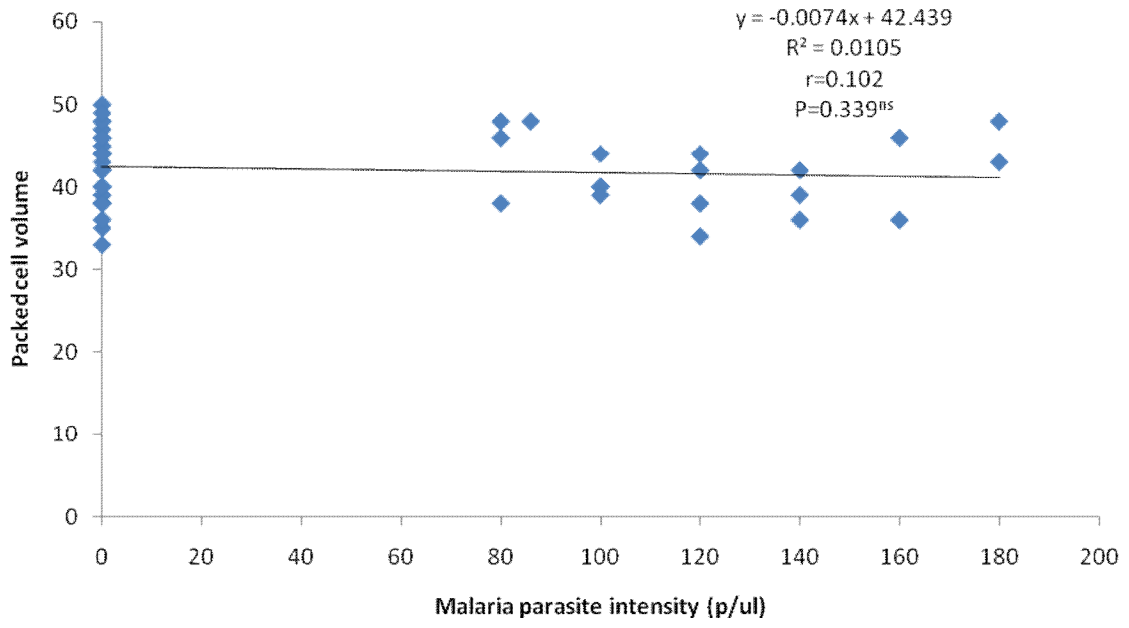

Fig. 14: Malaria Parasite Intensity in relation to Packed Cell Volume

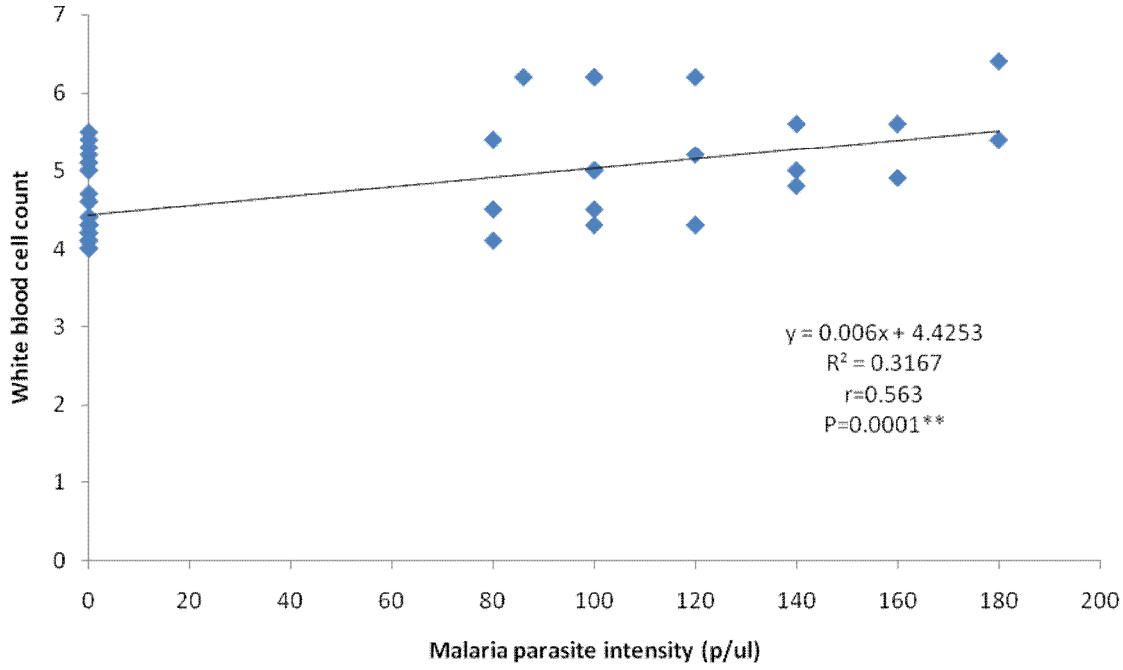

Fig. 15: Malaria Parasite Intensity in relation to White Blood Cell Count

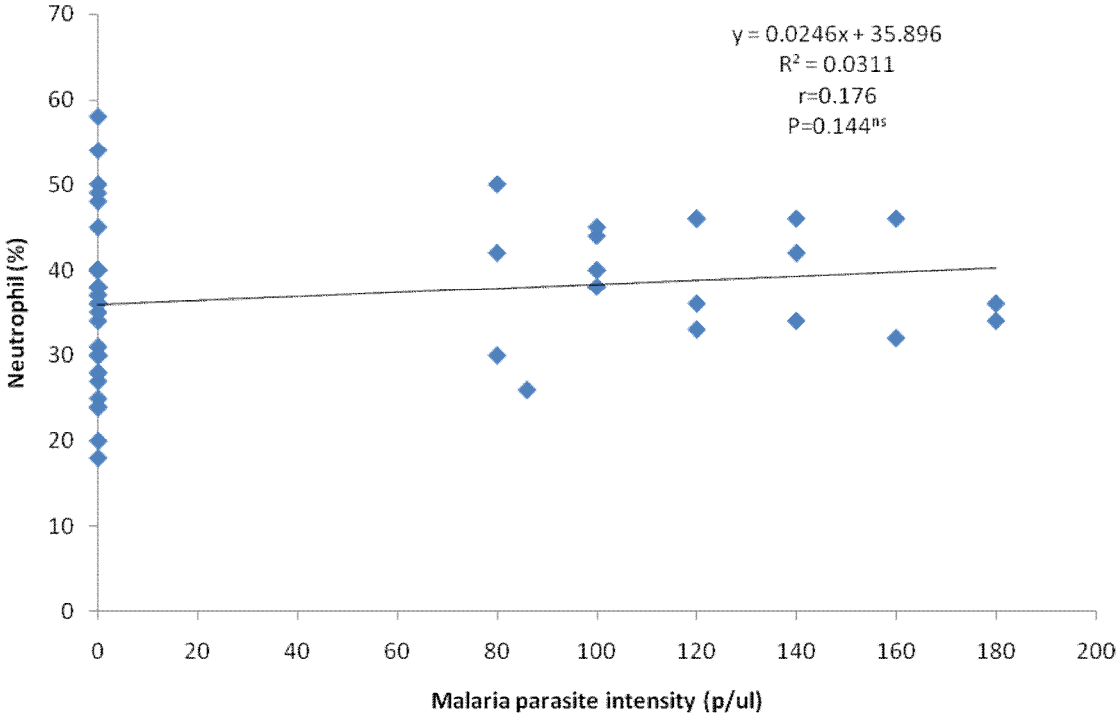

Fig. 16: Malaria Parasite Intensity in relation to Neutrophil Count

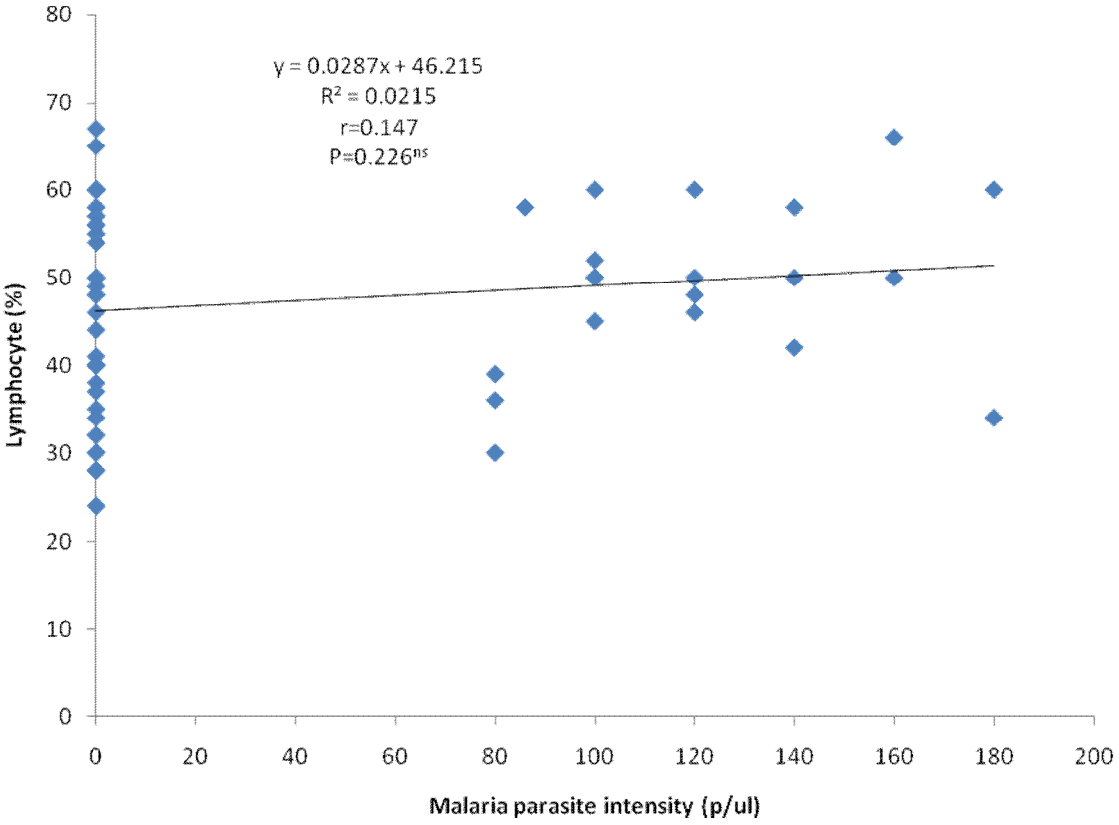

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Fig. 17: Malaria Parasite Intensity in relation to Lymphocyte Count

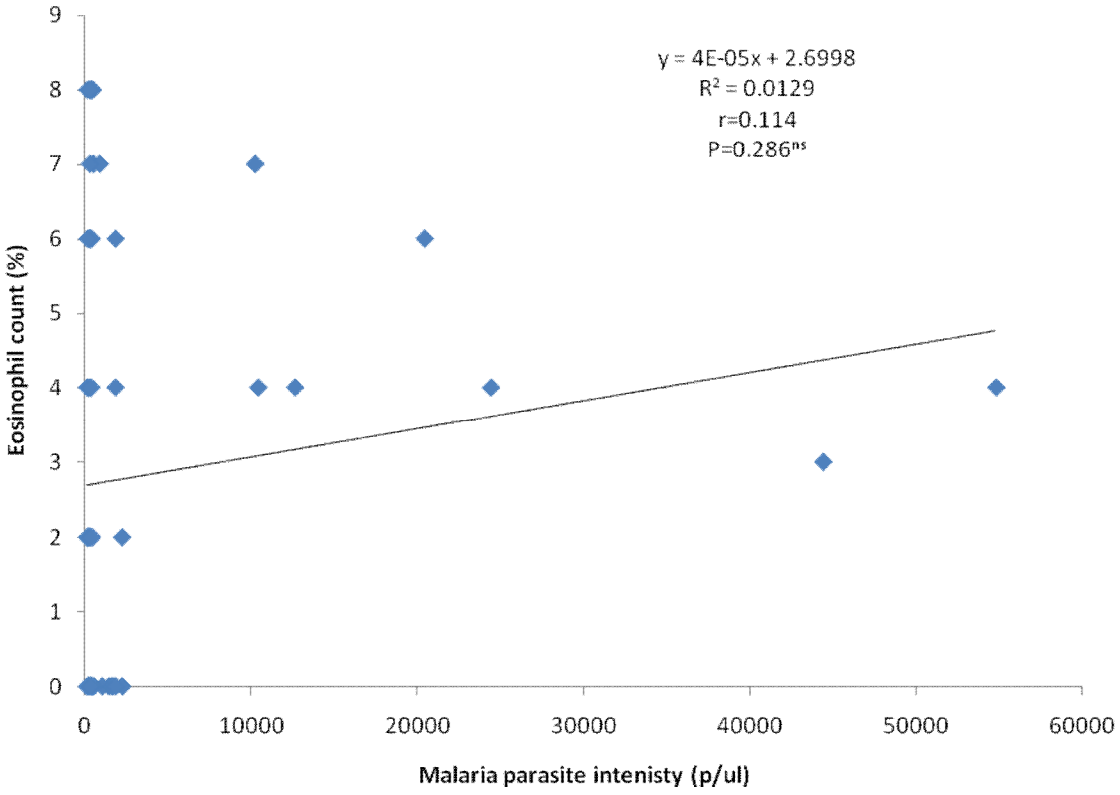

Fig. 18: Malaria Parasite Intensity in relation to Eosinophil Count

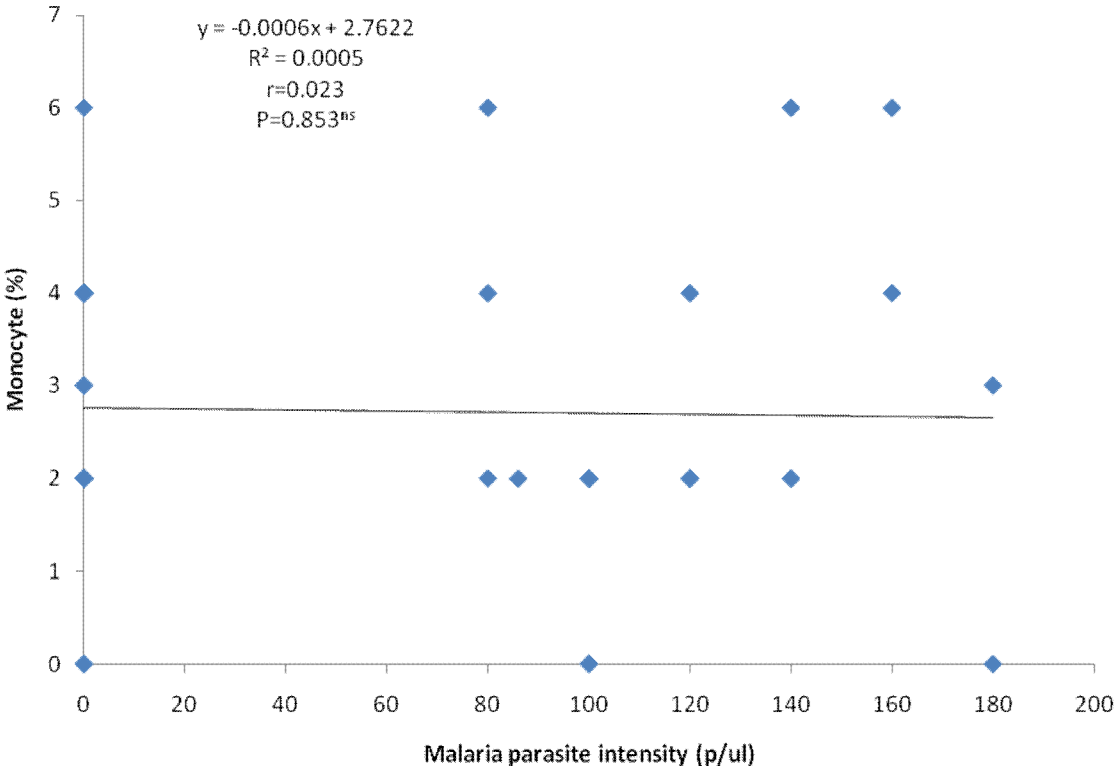

Fig. 19: Malaria Parasite Intensity in relation to Monocyte Count

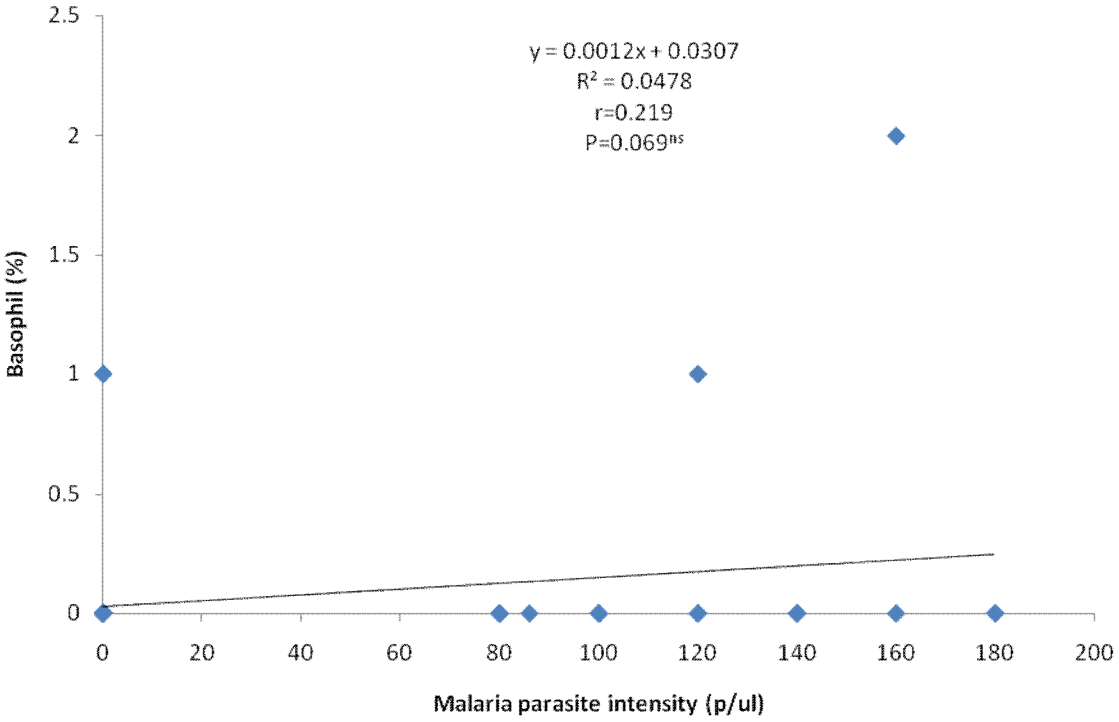

Fig. 20: Malaria Parasite Intensity in relation to Basophil Count

3.5.1: Haematological Parameters of Malaria Positive and Negative Individuals due to Sex.

The mean values of Total Leucocyte Counts, Neutrophils, Eosinophils and Basophils are higher in positive males than in the negative males. The values of Packed Cell Volume, Lymphocytes and monocytes are slightly higher in the negative males but the differences are not significant ($P > 0.05$). In the females, the mean values of Total Leucocyte Counts, Neutrophils, Lymphocytes and Eosinophils are higher in positive individuals than in the negative ones. Among the females also, the values of Packed cell volume, Monocytes and Basophils are higher in the negative individuals than in the positive ones (Table 13).

3.5.2: Haematological Parameters of Malaria Positive and Negative Individuals due to Age.

The mean PCV values of control individuals were higher in all age classes except in the class 59 – 65 years but the differences were not significant. In the case of TLC, the infected individuals had higher TLC values than the controls in all the age classes. The differences were statistically significant in the 10 -16 years age class. ($P < 0.05$) (Table 14). The levels of neutrophils were also higher in the malaria positive individuals than the controls in all the age classes except those of 3 – 9 years and 24 – 30 years. The differences, however, were not significant. The mean values of lymphocytes in the infected individuals were higher than the controls in age classes 3 – 9 years, 17 – 23 years, 24 – 30 years, 45 – 51 years and 52 – 58 years while the controls had higher values than their infected counterpart in age classes 10 – 16 years, 31 – 37 years, 38 – 44 years and 59 – 65 years. The differences, however, is only significant at age class 45 – 51 years (Table 14). The mean eosinophil levels of infected individuals were higher than those of the controls in all the age classes and

the differences were significant in the 31 – 37 years, 52 – 58 years and 59 – 65 years age classes (Table 14). In the mean levels of basophils, the infected individuals had slightly higher values in age classes 10 – 16 years, 24 – 30 years, 31 – 37 years and 59 – 65 years. In the remaining classes, both the controls and infected individuals had same values. In the mean monocytes levels, the controls had higher values than the infected individuals in all the age groups except in those of 24 – 30 years and 45 – 51 years where the controls had higher values. The differences were significant in the age groups 3 – 9 years and 17 – 23 years (Table 14).

3.6: Mean kidney Sizes of Malaria Positive and Negative Individuals.

The mean sizes of both right and left kidneys of malaria positive subjects were higher than those of the controls (Table 15). The differences were also significant.

Table 13: Sex Comparison of Haematological Parameters of Positive and Negative Individuals.

Sex	Heam. Parameters (%)	Positives (n = 45)	Negatives (n = 62)	P value
Male	PCV	36.64±4.71	41.94±3.49	0.007*
	TLC ($x10^9$/L)	6.08±1.72	5.18±0.64	0.004*
	Neut.	41.13±10.81	38.26±11.26	0.185^{ns}
	Lympho.	51.16±12.66	52.90±9.66	0.420^{ns}
	Eosinoph.	4.66±2.91	3.22±2.80	0.011*
	Monocy	3.09±2.02	3.89±2.54	0.74^{ns}
	Basoph	0.11±0.44	0.00±0.00	0.048*
Female	PCV	39.18±4.57	40.81±3.70	0.042*
	TLCs ($x10^9$/L)	5.70±0.93	5.39±0.69	0.027*
	Neut.	40.24±9.20	38.07±974	0.209^{ns}
	Lympho.	54.47±13.23	53.76±8.61	0.712^{ns}
	Eosinoph.	4.22±3.37	2.36±2.56	0.001*
	Monocy	3.56±1.63	4.28±2.41	0.070^{ns}
	Basoph	0.02±0.20	0.05±0.02	0.462^{ns}

* = significant difference between sexes of positive and negative individuals at (P<0.05)

ns = no significant difference (P>0.05)

Table 14: Age comparison of haematological parameters of Malaria Positive and Negative Individuals.

Age (yrs)	PCV			WBC			NEUT			LYM			EOS			BASO	
	Pos	Neg	P Value	Pos	Neg	P value	Pos	Neg	P value	Pos	Neg	P Value	Pos	Neg	P value	Pos	Neg
3 – 9	37.00±2.82	40.72±3.85	0.264^{ns}	5.50±0.42	5.22±0.64	0.52^{ns}	22.00±5.65	35.63±11.79	0.084^{ns}	61.50±20.51	58.54±7.16	0.684^{ns}	2.00±2.83	5.81±3.40	0.263^{ns}	0.00±0.00	0.00±0.00
10 – 16	38..29±6.28	41.64±4.03	0.139^{ns}	6.14±1.19	5.09±0.59	0.014^{ns}	44.43±4.29	38.18±10.82	0.214^{ns}	44.43±11.06	52.36±8.62	0.056^{ns}	3.36±2.87	4.91±3.94	0.287^{ns}	0.07±0.27	0.00±0.00
17 – 23	39.44±3.82	40.97±4.05	0.196^{ns}	5.47±0.68	5.39±0.71	0.691^{ns}	41.78±4.29	39.00±10.21	0.279^{ns}	51.33±10.98	50.52±7.09	0.753^{ns}	3.17±2.57	3.55±2.41	0.612^{ns}	0.00±0.00	0.03±0.18
24 – 30	39.96±5.11	41.24±3.52	0.285^{ns}	5.94±1.53	5.35±0.72	0.074^{ns}	40.00±10.76	42.07±10.02	0.461^{ns}	55.41±14.43	49.17±8.57	0.052^{ns}	3.00±2.90	3.66±2.78	0.392^{ns}	0.07±0.07	0.07±0.05
31 – 37	39.50±3.58	40.82±2.70	0.280^{ns}	6.64±2.29	5.39±0.60	0.021^{ns}	39.17±8.37	37.18±9.84	0.540^{ns}	51.50±12.09	56.55±7.63	0.209^{ns}	2.50±0.89	6.45±0.90	0.004*	0.17±0.17	0.05±0.05
38 – 44	39.25±4.57	41.62±3.22	0.386^{ns}	5.95±1.12	5.07±0.51	0.027^{ns}	43.75±13.53	34.50±11.66	0.275^{ns}	47.00±15.45	57.94±11.75	0.258^{ns}	3.25±2.99	3.44±2.92	0.915^{ns}	0.00±0.00	0.00±0.00
45 – 51	40.83±5.98	43.17±3.90	0.414^{ns}	4.92±0.34	5.39±0.52	0.035^{ns}	37.00±10.48	36.17±10.87	0.878^{ns}	68.83±3.37	54±75±10.10	0.012*	1.83±2.40	3.67±2.54	0.163^{ns}	0.00±0.00	0.00±0.00
52 – 58	38.25±0.96	40.22±315	0.117^{ns}	5.60±0.59	5.56±0.75	0.911^{ns}	40.50±4.38	40.78±7.79	0.937^{ns}	53.00±8.41	52.33±914	0.902^{ns}	1.00±1.16	5.44±2.19	0.001^{ns}	0.00±0.00	0.00±0.00
59 - 65	39.67±2.52	39.50±6.03	0.963^{ns}	5.27±1.01	5.05±1.11	0.809^{ns}	45.00±5.19	33.50±10.50	0.121^{ns}	56.33±10.12	57.00±5.29	0.924^{ns}	1.33±1.16	5.00±1.16	0.011^{ns}	0.33±0.33	0.00±0.00

* = significant difference (P<0.05)

Table 15: Mean kidney sizes of malaria positive and control individuals

Kidney	Positive	Negative	P- value
Right	3956.95 ± 133.995	3480.70 ± 110.52	0.009
Left	4133.50 ± 125.005	3624.20 ± 104.521	0.003

Table 16: Sex Comparison of Biochemical Parameters in Malaria Positive Individuals

Biochemical parameters	Male (n = 45)	Female (n = 45)	P = value
Total protein(mg/dl)	75.61±11.21	75.46±16.97	0.958^{ns}
Urea(mmol/L)	4.00±3.29	4.54±2.12	0.44^{ns}
Albumin(mmol/L)	44.76±6.86	46.91±5.73	0.110^{ns}
Creatinine(µmol/L)	101.75±30.32	82.87±19.63	0.001^{*}

ns = no significant difference (P>0.05)

* = significant difference (P<0.05)

Table 17: Age Comparison of Biochemical Parameters of Positive Individuals

Age(years)	Total Protein(mg/dl)	Urea(mmol/L)	Albumin(mg/dl)	Creatinine(µmol/L)
3 – 9	70.25 ± 13.78^{a}	3.70 ± 0.00^{b}	44.10 ± 0.14^{a}	80.25 ± 8.13^{b}
10 – 16	70.86 ± 10.24^{a}	3.81 ± 1.17^{b}	46.26 ± 6.20^{a}	90.61 ± 33.10^{b}
17 – 23	79.85 ± 24.54^{a}	4.54 ± 2.79^{b}	45.51 ± 7.21^{a}	90.59 ± 29.32^{b}
24 – 30	73.10 ± 8.62^{a}	4.57 ± 2.38^{b}	46.17 ± 5.93^{a}	89.32 ± 22.06^{b}

31 – 37	74.65±8.59[a]	5.04±2.75[ab]	45.07±6.47[a]	89.23±25.34[b]
38 – 44	75.53±9.61[a]	5.72±2.24[ab]	45.35±10.12[a]	107.30±21.84[ab]
45 – 51	86.47±14.41[a]	7.50±6.22[a]	45.10±4.17[a]	100.22±27.61[ab]
52 – 58	82.30±5.65[a]	5.25±1.53[ab]	44.60±2.25[a]	88.73±23.57[b]
59 – 65	88.26±14.29[a]	4.70±1.41[ab]	50.83±13.27[a]	142.60±41.57[a]

Mean values with different alphabets are significantly different (P<0.05)

3.7: Mean Biochemical Parameters of Malaria Positive Individuals due to Sex.

Table 16 shows that there were slight differences in the levels of total protein, urea, albumin and creatinine levels between the males and females with the males having higher levels except in albumin where the females had higher values. However, the differences were not significant except in the mean creatinine level.

3.8: Biochemical Parameters of Malaria Positive Individuals due to Age

Among the positive cases, age group 59 – 65years had the highest mean value of Total Protein (88.26±14.29 mg/dl) while 3 – 9 years had the least (70.25±13.78mg/dl). In the mean value of Urea, the age group 45 – 51 years had the highest mean value (7.50±6.22mmol/L). Age group 59 – 65 years had the highest

mean value of Albumin (50.83±13.27mg/dl) while the age group 3 – 9 had the lowest (44.10±0.14mg/dl). Mean creatinine level was also highest at age group 59 – 65 years (142.60±41.57µmol/L) and lowest at 3 – 9 years(80.25±8.13µmol/L) (Table17).

3.9: Serum Electrolytes of Malaria Positive Individuals due to Sex.

The mean levels of serum electrolytes is shown in Table 18 with only bicarbonate ions having significant difference in the sexes.

3.10: Serum Electrolytes values of Malaria Positive Individuals due to Age.

The age group 3 – 9 years had the highest mean value of sodium ion (142.0±1.419mmol/L) followed by the age group 52 – 58 years(139.75±3.30) while the age group 59 – 65 years had the least (133.33±1.53mmol/L). The mean value of potassium was highest in the age group 59 – 65 years (4.27±1.07mmol/L), followed by the age group 24 – 30 years (3.91±0.10mmol/L) while the age group 3 – 9 years had the least (3.25±0.05mmol/L). However, the difference was only significant between the age groups 3 – 9 years, 24 – 80 years, 45 – 51 years and 59 – 65 years (Table 19).

Table 18: Sex Comparison of Serum Electrolytes of Positive Individuals

Serum electrolytes	Male (n = 45)	Female (n = 45)	P = value
Sodium ion(mmol/L)	137.80 ± 3.29	137.02 ± 3.48	0.278^{ns}
Potassium ion(mmol/L)	3.62± 0.55	3.91 ± 0.62	0.021^{ns}
Chloride ion(mmol/L)	105.84 ± 3.79	105.87 ± 3.74	0.978^{ns}
Bicarbonateion(mmol/L)	24.22 ± 3.26	2.20 ± 3.88	0.005*
Specific Gravity	1.02 ± 0.03	1.02 ± 0.01	0.282^{ns}
pH	6.41 ± 1.00	6.11 ± 0.992	0.143^{ns}

ns= no significant difference (P>0.05)

*=significant difference (P<0.05)

Table 19: Age Comparison of Serum Electrolytes of Malaria positive Individuals.

Age(years)	Na^+(mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)	HCO_3^- (mmol/L)	SG	pH
3 – 9	142.0 ± 1.41^{a}	3.25±0.05^{c}	103.50±1.50^{ab}	26.0 ±1.00^{ab}	1.020±0.007^{a}	6.50±0.50^{ab}
10 – 16	135.86± 3.63^{bc}	3.57±0.21^{abc}	105.57±104^{ab}	21.79±1.22^{b}	1.020±0.001^{a}	5.67±0.17^{b}
17 – 23	137.44 ± 3.13^{ab}	3.74±0.09^{abc}	408.06±0.83^{a}	23.03±0.91^{ab}	1.030±0.009^{a}	6.50±0.22^{a}
24 – 30	137.10 ± 2.81^{bc}	3.91±0.10^{b}	106.26±0.73^{ab}	23.51±0.73^{ab}	1.020±0.001^{a}	6.50±0.21^{a}
31 – 37	138.75±3.62^{ab}	3.84±0.12^{abc}	105.50±1.18^{ab}	24.17±0.82^{ab}	1.020±0.002^{a}	6.33±0.31^{ab}
38 – 44	138.25±2.98^{ab}	3.87±0.10^{abc}	104.74±1.11^{ab}	21.75±1.49^{ab}	1.020±0.003^{a}	6.00±0.41^{ab}
45 – 51	137.23±4.26^{abc}	3.37±0.20^{c}	103.33±1.33^{b}	21.83±1.49^{ab}	1.020±0.002^{a}	5.75±0.36^{ab}
52 – 58	139.75±3.30^{ab}	3.85±0.23^{abc}	107.50±2.90^{ab}	26.0±1.08^{a}	1.030±0.001^{a}	6.25±0.14^{ab}
59 – 65	133.33±1.53^{c}	4.27±1.07^{a}	103.67±0.33^{ab}	21.33±1.45^{ab}	1.020±0.004^{a}	6.33±0.67^{ab}

Mean values with different alphabets are significantly different ($P<0.05$)

3.11: Haematological Parameters of Malaria Positive Individuals due to Sex.

There is no significant difference between the mean values of haematological parameters of males and females among the positive subjects(Table 20).

3.12: Age Comparison of Haematological Parameters in Malaria Positives

The mean PCV level is highest in the age class 45 – 51 years (40.83±5.98%), followed by the age class 24 – 30 years (39.96±5.711%) and least at age class 3 – 9 years (37.00±2.83%). The differences are not significant at (P > = 0.05) (Table 19). The mean values of TLC was highest in the age class 31 – 37 years (6.64±2.29x10^9/L) and least in the age class 45 – 51 years (4.92±0.34x10^9/L). The difference is significant between the ages 31 – 37 years and 45 – 51 years (Table 19). In the same way, the age class 59 – 65 years had the highest mean value of neutrophil (45.00±5.19 %) while the age class 3 – 9 years had the least (22.00±5.65%) (Table 19). The mean Lymphocyte value is highest in the age class 45 – 51 years (66.83±3.7%) and least in the age class 10 – 16 years (44.43±11.06%). Eosinophil value is highest in the age class 10 – 16 years (3.36±0.77%) and least in age class 52 – 58 years (1.00±0.58%). Mean value of monocytes is highest in the age class 24 – 30 years (3.89±0.36%) and least in the age class 3 – 9 years (2.00±00%). Basophil mean value is highest in the age class 59 – 65 years followed by the age class 31-37years but the differences were not significant (Table 21).

Table 20: Sex Comparison of Haematological Parameters of Infected Individuals.

Haematological Parameter	Male (n = 45)	Female (n = 45)	P = value
Packed cell volume(%)	39.64 ± 4.71	39.18 ± 4.57	0.635^{ns}
Total leucocyte count($x10^9$/L)	6.01 ± 1.72	5.70 ± 0.93	0.301^{ns}
Neutrophils(%)	41.13 ± 10.81	40.24 ± 9.20	0.675^{ns}
Lymphocyte(%)	51.16 ± 12.66	54.47 ± 13.22	0.228^{ns}
Eosinophils(%)	3.22 ± 0.42	2.36 ± 0.38	0.129^{ns}
Monocytes(%)	3.09 ± 0.30	3.56 ± 0.24	0.231^{ns}
Basophils(%)	0.11 ± 0.06	0.02 ± 0.02	0.089^{ns}

ns = no significant difference (P>0.05)

Table 21: Age differences in Haematological Parameters of malaria positive Individuals

Age(years)	PCV(%)	TLC(x10^9/L)	NEUT(%)	LYMPH(%)	EOS(%)	MONO(%)	BASO(%)
3 – 9	37.00 ±2.83^{a}	5.50±0.42^{ab}	22.00±5.65^{b}	61.50±20.51^{ab}	2.00±2.00^{a}	2.00±0.00^{b}	0.00±0.00^{a}
10 – 16	38.29±6.28^{a}	6.14±1.19^{ab}	44.43±13.62^{a}	44.43±11.06^{c}	3.36±0.77^{a}	3.07±0.57^{ab}	0.07±0.07^{a}
17 – 23	39.44±3.82^{a}	5.47±0.68^{b}	41.78±4.29^{a}	51.33±10.98^{bc}	3.17±061^{a}	2.61±0.41^{b}	0.00±0.00^{a}
24 – 30	39.96±5.11^{a}	5.94±1.53^{ab}	40.00±10.76^{a}	55.41±14.43^{b}	3.00±0.56^{a}	3.89±0.36^{a}	0.07±0.07^{a}
31 – 37	39.50±3.58^{a}	6.64±2.29^{a}	39.17±8.36^{a}	51.50±12.09^{bc}	2.50±0.89^{a}	3.75±0.45^{ab}	0.17±0.16^{a}
38 – 44	39.25±4.57^{a}	5.95±1.12^{ab}	43.75±13.53^{a}	47.00±15.45^{bc}	3.25±1.49^{a}	2.50±0.76^{b}	0.00±0.00^{a}
45 – 51	40.83±5.98^{a}	4.92±034^{b}	37.00±10.4^{ab}	66.83±3.37^{a}	1.83±0.98^{a}	3.67±0.80^{ab}	0.00±0.00^{a}
52 – 58	38.25±0.96^{a}	5.60±0.59^{ab}	40.50±4.43^{a}	53.00±8.41^{abc}	1.00±0.58^{a}	3.75±085^{ab}	0.00±0.00^{a}
59 – 65	39.67±2.52^{a}	5.27±1.10^{ab}	45.00±5.19^{a}	56.33±10.12^{abc}	1.33±0.61^{a}	2.67±0.67^{b}	0.33±0.33^{a}

Mean values with different alphabets are significantly different (P<0.05).

3.13: Prevalence of Malaria Parasite After Treatment

The prevalence of MP in the 70 individuals that co-operated/subjected themselves to treatment before drug administration was 31.8%. After treatment, only 9.1% (n = 20) were positive for the infection.

3.13.1 **Categories of different levels of MP before and after treatment.**

The mean intensity of malaria infection before treatment 2759.71p/µ was quite higher than that after treatment (34.37 ±6.85). The difference is statistically significant ($P < 0.05$) (Table 22).

3.13.2: Sex and Age Prevalence of Malaria after treatment.

After treatment, more males (11) were infected than females (9) but the difference was not significant. According to age, the highest infection 7(14.3) occurred at the age class 24 – 30 years while the least occurred at the age classes 31 – 37 and 52 – 58 years (Table 23). The differences, however, were not significant ($P > 0.05$).

3.14: Biochemical Parameters of malaria positive Individuals before and after treatment.

The mean levels of all the biochemical parameters (total protein, urea, albumin and creatinine) were reduced after the treatment of malaria positive individuals. The differences between the treated and untreated individuals were highly significant. ($P < 0.01$) (Table 24).

3.15: Mean Serum electrolytes of Malaria Positive Individuals before and after treatment

The mean values of electrolytes were reduced after treatment of the infected individuals except the pH. The differences between sodium, potassium, chloride and bicarbonate ions are highly significant while those between specific gravity and pH are not significant (Table 25).

3.16: Haematological Parameters of Malaria Positive Individuals before and after treatment.

Apart from the mean value of PCV, the mean values of other parameters reduced after the treatment of malaria positive individuals. The differences were also significant except in eoesinophils and basophils (Table 26).

Table 22: Categories of different levels of MP after treatment as compared to before treatment

Categories (%)	(n = 70) untreated(%)	(n = 70) Treated(%)
Mild	56 (80)	20(28.6)
Moderate	9(12.9)	0(0)
Severe	5(7.1)	0(0)
Mean intensity	2759.71	34.37 ±6.85

P value = 0.014*

Table 23: Sex and Age prevalence after treatment

	Number	n(%)
Over all Sex	220	20(9.1)
Male	96	11(11.5)
Female	124	9(7.3)
x^2		1.155
P value		0.282^{ns}
Age (years)		
3 – 9	12	0(0)
10 – 16	22	2(9.1)
17 – 23	48	5(10.4)
24 – 30	49	7(14.3)
31 – 37	32	1(3.1)
38 – 44	19	2(10.5)
45 – 51	16	1(6.23)

52 – 58	10	0(0)
59 65	7	2(28.6)
66 – 72	5	0(0)
x^2		9.199
P value		0.419^{ns}

Table 24: Comparison of Biochemical Parameters before and after Treatment

Biochemical parameters	(n = 70)	(n = 70) Untreated	P value
TP(mg/dl)	49.38±8.29	73.46±15.32	0.0001^{**}
Urea(mmol/L)	2.99±0.86	4.74±6.99	0.0001^{**}
ALB(mg/dl)	33.91±5.33	46.62±6.92	0.0001^{**}
Creatinine(µmol/L)	55.47±11.32	94.73±26.70	0.0001^{**}

** High significant difference (P < 0.01)

Table 25: Mean Serum Electrolytes of Malaria Positive Individuals before and after Treatment.

Serum electrolyte	(n = 70) Treated	(n = 70) Untreated	P value
Sodium(mmol/L)	128.60±3.57	137.87±3.43	0.0001^{**}
Potassium(mmol/L)	2.82±0.45	3.78±0.65	0.0001^{**}

Chloride(mmol/L)	103.31±3.03	106.37±3.54	0.0001**
Bicarbonate(mmol/L)	20.38±2.60	22.52±3.80	0.0001**
Specific Gravity	1.02±0.03	1.03±0.02	0.273ns
PH	6.07±1.14	6.20±1.04	0.467ns

** High significant difference (P < 0.01)

ns no significant difference (P > 0.05)

Table 26: Haematological Parameters of Malaria Positive Individuals before and after treatment.

Haematological parameter(%)	(n = 70) Treated	(n = 70) Untreated	P value
PCV	42.19±4.13	39.26±4.88	0.0001**
TLC(x10^9/L)	4.63±0.61	5.80±1.32	0.0001**
NEUT	36.74±7.9	41.09±8.32	0.002*
LYMPH	47.20±11.19	52.40±12.56	0.011*
EOSINO	2.16±1.72	2.74±2.65	0.123ns
MONO	2.74±1.43	3.39±1.89	0.025*
BASO	0.07±0.04	0.09±0.07	0.805ns

** High significant difference (P < 0.05)

* Significant difference (P< 0.05)

ns no significant difference (P > 0.05)

CHAPTER FOUR

4.0 DISCUSSION

4.1: Prevalence of malaria Parasitaemia in the Study Area

The result of this study showed that the overall prevalence of *Plasmodium* parasite was 37.50% (n=90). This indicated that malaria is endemic in the area as it is in every other part of the country. The finding agreed with several reports from both within and outside the country (Salako *et al.,* 1990, Ademowo *et al .,* 1995, Afolabi, 1997, Nwaorgu and Orajiaka, 2011 and Kalu *et al*., 2012). These researchers all reported high prevalent rates in their respective studies and this agreed with Ukpai and Ajoku (2001) that malaria infection is holo-endemic in Nigeria and widespread in tropical and subtropical regions of Africa, Asia and Americas. These high rates can be one of the reasons for high mortality rates in these areas especially in children and pregnant women. Infection is acquired wherever there are human hosts

carrying the parasites and sufficient *Anopheles* mosquitoes, together with conditions of temperature and humidity that favour the development of parasites in the mosquitoes. Such factors are readily available in the tropics, hence, the high prevalent rates in these regions. However, the prevalent rate of malaria parasitaemia in this present study is much lower than the reports referred to above and those of (Sowunmi, 1996, Seboxa and Snow, 1997 and Adeyemo *et al.,* 1999) who reported 74.1%, 68% and 80% parasitaemia respectively. The wide differences between the result of the present study and those of earlier studies may be attributed to the increased awareness about the infection and the increased use of insecticide treated nets and similar measures in the prevention of malaria.

Gender-wise, the study showed that there was no sex difference in infection as same number of both sexes was infected. This disagreed with studies by Okeahialam, *et al;* 1972, Mbanugo and Ejim, 2000, Uzoegwu, and Onwurah, 2003, Ani, 2004, Nwaorgu and Orajiaka 2011 and Okafor and Oko-Ose, 2012). All these researchers reported higher prevalences in males than females. This could be due to the fact that the males expose themselves more than the females especially during hot weather. At such times, they tend to move about bare-bodied and expose themselves more to mosquito bites than the females. Krogstad, (1996) also attributed such sex differences in malaria infection to genetic and hormonal factors. However, in most of the studies that reported sex differences, such differences were not statistically significant. The present study is, however, in agreement with the findings of Mbanugo and Ejim (2000) who reported that sex did not affect the prevalence of malaria.

In the present study, the age range 10-16 years had the highest prevalent rate of 56.0% followed by 24-30 years while the age range 66-72 years had no

infection. This is also similar to the findings of Aribodor *et al*., (2003), Nwaorgu and Orajiaka ,(2011) which all reported decline in prevalence by age. This also agreed with the report of Ani (2004) that children in the first decade of life had the highest prevalence of malaria. The most likely reason for decline in prevalence by age is that older individuals may have developed anti-malaria immunity after many years of chronic expose to mosquito bites and malaria infection. Immunity has important effects on the transmission of the disease by reducing the level of parasitaemia after infective bites and increasing ten folds the rate of clearance of parasitaemia (Ademowo,1995). *Plasmodium* infection was more prevalent in younger individuals because of their relatively less developed immunity to the infection than the older individuals.

Analysis of data on the categories of malaria parasite prevalence in this study, revealed that majority of the infected individuals had mild infection. For example, 70 (77.8%) out of 90 positive individuals had mild infection (Table 2). Furthermore, the mean parasite density of the 90 positive individuals is 2361.89 $\pm$ 857.55p/µl. This shows that majority of the infected individuals had low parasite densities. This could be attributed to natural immunity derived by these individuals from persistent attacks of malaria. This is in agreement with previous findings of Ogunrin (2001) who stated that in hyper-endemic areas, the disease is mild and asymptomatic especially in the adults. Therefore age and nutritional status of the host may represent natural or acquired resistance and hence can play a role in the severity of the disease produced.

4.2: Biochemical Parameters of Malaria Infected Individuals and the Controls.

The study revealed higher values of total protein, urea, albumin and creatinine in malaria positive individuals than the uninfected ones. The differences were statistically significant except in albumin levels. The infected individuals had higher mean concentration of protein (75.54 ± 14.30mg/dl) than the control group (71.07 ± 10.53mg/dl) and differences were statistically significant at $P<0.05$. This confirms the earlier reports of (Ogbadoyi and Tembeg, 1999; Ogbadoyi and Gabi, 2000; Mishra *et al*., 2002; Ekeanyanwu and Ogu, 2010; Idonije *et al*., 2011; and Akaninwor *et al.,* 2013). High level of proteinuria is an indication of renal impairment (Rui-mei *et al.*, 1998). Proteins are normally filtered completely and reabsorbed from the blood stream of healthy kidneys, allowing no protein or only untraceable amounts into the urine. When a reasonable amount is persistently present in the urine, it is a useful indicator of a form of kidney disease. The higher level of protein in the serum of infected people in this study can be attributed to malaria as there was positive correlation between proteinuria and level of malaria parasitaemia ($r=0.18$). However, it is worthy of note that conditions such as orthostatic proteinuria in healthy children and asymptomatic bacteria infection may also contribute to high excretion of protein in the urine (Edwards and Boucher, 1991; Ogbadoyi and Tembeng, 1999). The infected individuals had higher mean concentration of serum urea (4.76±2.76mmol/L) than the control group (4.08±1.69mmol/L) and the difference was statistically significant. There was also positive significant association between malaria infection and serum urea $r=0.348$. This corroborates the reports of, Ogbadoyi and Tsado, 2009 and Ekeanyanwu and Akpoilih, 2010. However, worthy of note is the fact that plasma urea level is also affected by a number of non-kidney related factors such as dehydration, food intake and tissue catabolism (WHO, 2000b). The mean serum albumin concentration of malaria patients (45.84±6.58mg/dl) was higher than that of

the control group (44.72±5.16mg/dl) but the difference was not statistically significant at p>0.05. This corroborated the reports of Crawly, 2004; Ogbodo *et al.*, 2010 and Amah *et al*., (2011). In the same vein, the mean serum concentration of creatinine (92.54±27.09µmol/L) in the infected subjects was significantly higher when compared to that of their control counterparts (p<0.01). Malaria parasitaemia and serum creatinine were also positively correlated with r=0.602. These findings are comparable to those of (Prakash *et al*., 2003; Bagshaw *et al*., 2008; Ogbadoyi and Tsado, 2009; Ekeanyanwu and Ogu 2010 and Akaninwor *et al*., 2013). Serum urea and creatinine concentration are used for the assessment of renal efficiency, hence when their values are higher than normal, deficiency in renal function is suspected. Elevation of plasma creatinine concentration could be suggestive of ineffective filtering ability of the kidneys which could lead to renal function impairment. Among the malaria positive individuals, no significant difference was observed in serum urea, albumin and protein in the urine due to sexes. This contrasted with the reports of (Melita *et al.*, 2001; Prakash *et al*., 2003 and Naqvi *et al*., 2003). Serum creatinine level on the other hand was significantly higher (101.75±30.32µmol/L) in the positive males than in the females (82.84±19.63µmol/L) (p<0.05). As high serum creatinine level is normally an indicator of inefficiency in renal function, sex difference in susceptibility to renal dysfunction in patients with falciparum malaria is possible. According to ages of the infected individuals, those between 59 - 65 years old had the highest total urine protein (88.26±14.29mg/mol) while those between 3 - 9 years old had the lowest (70.25±13.78mg/dl). The observation is that the older individuals had higher total protein levels than the younger ones but this was not statistically significant. This is in contrast to a previous report of higher protein level in younger individuals by Ekeanyanwu and Akpoilih (2010). There

was also no significant difference in the serum levels of albumin. On the other hand, the differences in the serum levels of creatinine and urea among the younger and older individuals were significant. As creatinine and urea are the main markers for renal impairment, it could be deduced that age may be a factor in renal impairment due to malaria parasitaemia.

4.3: Serum Electrolytes in Malaria Positive and Control Individuals.

In this study, the levels of all serum electrolytes in malaria positive and negative individuals showed no significant differences ($p>0.05$.) This is in consonance with previous works done by Ekeanyanwu and Akpoilih (2010) at Owerri, Imo State. Ogbadoyi and Gabi, (2010) also recorded no significant difference in sodium levels between the males of malaria patients and controls, but significant variations were found in sodium levels of female malaria patients and the controls ($p<0.05$). Significant differences also existed in potassium levels of male malaria individuals and the controls while in the level of bicarbonate, there was no significant difference between the positive and negative individuals ($p>0.05$), (Adesina *et al*., 2009; Ogbodo *et al*., 2010; Kayode *et al*., 2011). In the infected subjects, significant difference was found only in the value of bicarbonates ($p<0.05$). The elevated serum bicarbonate level in the present study may primarily be due to factors other than malaria as

there was no positive correlation between parasitaemia and bicarbonate levels.

4.4: Haematological Parameters of Malaria Infected and Control Individuals.

Haematological changes have been associated with malaria infection and these have been found to involve red blood cells, leukocytes and thrombocytes (Layla *et al*., 2002; Maina *et al.,* 2010; Imoru *et al.*, 2013). In this study, significantly lower values of PCV and monocytes were observed in malaria infected individuals compared to the controls. This is in agreement with the findings of previous studies of (Trape, 1985; George and Ewelike-Ezeani, 2011). The reduction in the PCV values in malaria positive subjects was earlier reported to result from the mechanical destruction of parasitized red blood cells, reduced red blood cell production in the bone marrow, phagocytosis of uninfected red blood cells and autoimmune destruction of red blood cells (Erhart *et al*., 2004). This could connote mild anaemia. These are some of the mechanisms that cause anaemia during malaria. The lower values of monocytes in the infected subjects corroborated the report of Ladhani *et al.*, (2002) which associated low values of monocytes with severe malaria. It was suggested that the function of monocytes may be inhibited by the action of hemozoin from digested haemoglobin (Weatheral *et al.*, 2002). The values of total leucocyte counts, neutrophils, eosinophils and basophils were higher in the infected subjects than the controls but the differences were only significant in the values of total leucocyte count and neutrophils. The result also showed significant positive correlation between malaria parasitaemia and the leucocyte counts (r=0.563). The significant higher values of total leucocyte count in the infected subjects contradicted the study of (Erharst *et al*., 2004; Smita and Harish, 2013 and Igbeneghu and Odaibo, 2013) which showed

significant lower values of total leukocyte count in malaria positive individuals. However, the present study is in agreement with the findings of (Perrin *et al*., 1982; Rojansthien *et al.*, 1992 and Ladhani *et al.*, 2002). Kayode *et a*l., (2011) also reported significant increase in total white blood cell count of malaria patients and posited that it could have been elicited by increased production of leukocytes at the onset of the infection to ward off malaria parasites. Similarly, increase in WBC in pregnant and non pregnant malaria patients has been reported by Adesina *et al.*, (2009). Among the infected individuals, there was no significant difference in haematological indices between males and females (Table 10). Therefore sex does not determine the level of these parameters in the infected persons. Among the age groups of infected persons, no significant difference was observed in the serum levels of PCV, eosinophils and basophils. In the level of total white blood cell count, significant variation was observed in 17 - 23 years old and 45 - 51 years old. In neutrophil levels, significant difference was observed only in the age group 3 - 9 years. From these results therefore, age is not a strong contributor to changes in haematological indices in malaria.

The ultrasonographic examination of the kdneys of both infected and control individuals revealed that the kidneys of malarial infected individuals were larger than those of the controls. It was also observed that the left kidneys were affected more than the right kidneys. The differences were significant in both kidneys. Some of the kidneys of malaria individuals were also observed to have lost cortico-medulary difference.

4.5: Malaria Parasite Prevalence after Treatment.

Treatment of malaria positive persons who submitted themselves to treatment reduced the prevalence from 31.8%(n=90) to 9.1% (n = 20). In the categories of infection, most of the people that still had malaria parasites in their peripheral blood after treatment had mild infection and the mean intensity dropped from 2759.71p/µl to 34.37±6.85p/µl. This reduction in prevalence also cut across sexes and ages as there was no significant differences in any of them.

Reduction in the levels of all serum biochemical parameters was observed after treatment of infected persons. The differences in their levels were all highly significant. Significant reduction in serum levels of sodium, potassium, chloride and bicarbonate ions were also observed. As biochemical indices and serum electrolytes are good indications of renal impairment, it could be implied that treatment of malaria in acute renal impairment with appropriate antimalarial chemotherapy will invariably reduce case fatality rates and enhance the recovery of renal function (Molta *et a*l.,1991; Perez *et al*., 2008). This agrees with the report of Naqvi, 2003. Star,1998; Mishra *et al*., 2002; Prakash *et al*., 2003; Idonije *et al;*, 2011and Das, 2008 all reported that malarial treatment with appropriate antimalarial drugs in addition to maintenance of fluid and electrolytes are some of the mainstay treatment in malarial acute renal failure. Therefore, early and prompt diagnosis along with antimalarial therapy are the main measures likely to reduce malarial acute renal impairment. There was also reduction in the levels of neutrophils, lymphocytes, eosinophils, monocytes and basophils but that of eosinophils was not significant. These cells are involved in phagocytosis and induction of immunity during infections. Therefore the reduction in their levels after treatment is in order. The packed cell volume was significantly increased after treatment. The level of packed cell volume has been reported to have drastically reduced during malaria infection (Imoru *et al*., 2013 and Smita and

Harish, 2013). Therefore after treatment and clearance of most of the malaria parasites, the level of the PCV improved. This agreed with the findings of Akaninwor *et al*., 2013 and WHO, 2005.

4.6: Findings

- Malaria prevalence is high in the study area as in every other part of the country in particular and the tropical region in general.
- Sex did not determine the trend of malaria parasite infection in this study
- There was decline in prevalence as age increased.
- Greater percentage of the infected individuals had mild infection which showed that malaria is both endemic and well tolerated in the study area.
- Malaria parasitaemia raised the values of biochemical markers of renal impairment as compared with their control counterparts showing that it is implicated in acute renal impairment.
- Malaria parasitaemia also raised the values of serum electrolytes in infected subjects (individuals) as compared with the controls but that was not significant.
- Malaria parasitaemia affected the haematological indices of the affected individuals
- Malaria parasitaemia also increased the kidney sizes and affected the cortico-medullary differentiation of positive individuals.
- Treatment of malaria infected individuals with anti-malarial drugs restored the levels of biochemical parameters, serum electrolytes and haematological parameters of the infected persons.

4.7: Recommendations

The prevention of malarial infection and early diagnosis are the measures likely to decrease malarial acute renal failure in developing countries. Early referral to centers equipped to provide renal replacement therapy, if necessary, along with anti malarial therapy and support could further reduce mortality and enhance recovery of renal function. Dehydration, if present, should be corrected carefully avoiding excessive fluid administration in order to minimize the rise of pulmonary oedema.

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