IMPLICATION OF MALARIA IN KIDNEY DYSFUNCTION

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DEDICATION

This work is dedicated to my parents. May ALLAH cover them with his blessings.

CERTIFICATION

This thesis entitled "Implication of malaria in kidney dysfunction " was carried out by Baba Gabi under my supervision and has been examined, read and found to meet the regulations governing the award of the degree of Masters of Technology in Biochemistry of the Federal University of Technology, Minna, and is approved for its contribution to knowledge and literary presentation.

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DECLARATION

I hereby declare that this thesis is my original work and has never to the best of my knowledge been submitted before for the award of any degree. All literature used in the course of this study has been duly cited in the reference.

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GABI BABA Date

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ABSTRACT

Renal failures do occur as a complication in about 1% of malaria cases, with higher mortality rate (about 45%). However, the level of awareness of its association with malaria is far from being satisfactory. As a contribution towards this awareness, this research project was designed to determine any correlation between malaria incidence and renal dysfunction in an endemic area like Minna. Renal function parameters, which include proteinuria, serum levels of urea creatinine and electrolytes (Na^+ ,K^+, HCO_3^- and Cl^-) were determined in 202 patients attending Federal University of Technology, Minna clinic and General Hospital, Minna and 58 non malaria individuals. The results obtained were analyzed statistically by ANOVA, correlation matrix and regression analysis based on the level of infection, sex and age group. Significant variations were obtained between proteinuria of patients ($p<0.05$) and that of the healthy individuals, and the female patient children showed the highest significant difference (mean; 33.87 ± 6.48). Positive correlation was noted between proteinuria and parasitaemia. The level of serum urea in female patient was significantly different (mean: 5.54±0.79, $P<0.05$) from that of healthy individual (mean: 4.96±0.22). creatinine level was statistically insignificant in both sexes , but positively correlated with urea . Sodium level showed significant variation between the female patients (mean: 133.65±1.34,$P<0.05$) and the healthy individuals (mean: 126.73±1.89). The potassium level of the male patients was significantly different (mean: 4.01±0.07, $P<0.05$) from that of healthy individuals (mean: 4.33±0.79). Other parameters were considerably insignificant. . From the variations noted in proteinuria , urea, creatinine, potassium and . Sodium levels, possible renal involvement in malaria cases could be inferred, which is at increase, with female children more susceptible to renal dysfunction in malaria infestation.

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CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUTION

One hundred and six years ago Ronald Ross described how mosquitoes carry malaria and hoped that the disease would soon be eliminated. However, it is one of the most serious health problems facing the entire world. In fact it emerged as one of the top killer disease in the world with record as the major cause of mortality in the tropical and subtropical regions (Roberts and Campbell, 1997; Carrington, 2001).

Nearly half the world population is vulnerable to malaria infection, about five hundred million people suffer from malaria, leading to death in 2 to 3 million cases annually [Mishra et al; 2002].Majority of the cases as well as deaths occur in sub-Saharan Africa. Out side Africa, the disease is seen in about 100 countries with Indian subcontinent and Brazil contributing two third of these cases (Mishra et al 2002).

Virtually no reliable statistics exist in Africa. However, an estimated 90% of the total malaria incidence and deaths is said to occur in Africa, particularly amongst pregnant women and children (Luxemburger, 1997; Carrington; 2001). Malaria is the most wide spread parasitic disease and one of the vector borne disease placed on world health organization's Disability Adjusted Life Years (DALYS) list, with falciparum malaria responsible for more death than any other type of the disease. (Carrington, 2001).

Resistance of the parasites to drugs and anopheline mosquitoes to insecticides are serious problems in its control. [Meek: 1997]. A serious complication associated with malaria is nephritic syndrome characterized by a marked Oedema [Cheesbrough, 1991a] proteinuria [Houba, 1979; Boonpucknaviq and Sitprija, 1979 and Rui-mei et al, 1998b], hyper kalaemia and metabolic acidosis [Mishra; 2002]. It occurs more frequently in children [Abdulrahman et al 1983a] and could progress to kidney damage due to inflammation of the glomerular basement membrane following the deposition of antigen-antibody complexes [Houba, 1979; Van velthuysen; 1996]. It finally results in renal failure, if the syndrome remains untreated for a long time.

Kidney is the central organ that carries out some vital homeostatic mechanisms which helps to maintain constant physiological parameters in the body. These parameters include the electrolyte contents of the body, normal acid- base balance of the body fluid, and the retention of vital substances e.g. glucose, amino acids and protein vital for body economy. It is also involved in the maintenance of the tonicity of body fluids by regulation of the water content of the body. Kidney is also involved in the removal of waste metabolic products such as urea, ammonia and creatinine [Gray and Haworth, 1982; Sheryl and Stark, 1997]. Kidney malfunction or failure results in homeostatic imbalances that could result in serious ill health or sudden death. This and other malaria associated

complications do possibly account for the higher morbidity and mortality rate that accompanies malaria infection.

1.2 BRIEF HISTORICAL BACKGROUND

Malaria is an acute infection caused by the protozoan parasite, plasmodium, transmitted by the mosquitoes of the genus Anopheles. Intermittent febrile attacks as well as splenohepatomegaly; anemia, lesions of the nervous system; kidney and other organs are characteristic features of the infection [Kreier, 1980; Encyclopedia Britannica, 1988].

The history of malaria is as old as man's history. It is the most ancient infection known and clearly defined, due to its peculiar clinical nature [Polozok, 1989]. The earliest record of malaria was 5th century BC [460-377 BC] as found in the record of the Hippo crates works. Malariology began in 1640 when Huandel Vego employed tincture of cinchona tree back for malaria treatment [Dulbecco; 1991]. The German scientist, Meckel, [1847] detected leukocytes-macrophages and brownish pigments associated with malaria. Alphose Laverai, [1880] an army surgeon in Algeria described malaria parasite in human red blood cell [RBC] while Golgi, [1885] established the presence of *plasmodium vivax* and *P. Malariae*. Sakharou, [1819]; Marchiafava and cell, [1890] described *P.falciparium*. Ronald Ross [1897] established that mosquitoes serve as vectors of human and avian malaria. Grassi Bignami Bastianelli confirmed this in 1898. Manson [1900] confirmed the mosquitoe's genus as anopheles. All as cited in Polozok, 1989.

In Africa, Staphenus [1922] discovered *P. ovale*, the fourth type of the causative agent of human malaria [WHO, 1983]. A recent finding shows that malaria is the biggest killer in human history, starting from as early as about 8000 BC [Connor, 2001]. Its incidence, rapid infiltration and geographical distribution were attributed to the birth and expansion of agricultural practices, which led to new mosquitoes-breeding sites. A study on human genes that confer limited immunity against the malaria parasite shows that a mild form of the disease arose in tropical Africa about 10,000 years ago. But it took a more lethal turn in the Middle East after the introduction of the farming techniques [Connor, 2001; Dobson and Snow, 2000; Malaria 2000]. Although the toll of morbidity and mortality that currently takes on African are well published, very few critical historical works are found. The significance of such historical finding could be envisaged on how the advent of the illness shaped human history and could also accounted for the genetic transformations that brought about some level of adaptation in surviving the fatal effect of the malaria illness. Such transformation was reported in a research on mutation in humans of the "house keeping gene" called Glucose-6-Phosphate dehydrogenase [G6PD] [Connor, 2001] that brought about limited protection against infection with plasmodium parasite [Cheesbrough, 1991a: Robinson, 2000 and Connor, 2001]. The mutation was reported to have arisen at about the same time that malaria became prevalent [Thomson and Connor, 2001].

Sickle haemoglobin and haemoglobin F in foetus are also associated with limited protection in human against malaria and are manifested in human as a result of genetic transformation [Cheesbrough, 1991a;]. Such an advent may possibly have historical relationship with malaria outbreak.

1.3 Aetiology

Malaria is caused by protozoan parasite that belongs to the class of the sporozoa; the order of haemosporidia; family of the plasmodidae; genus plasmodium. Over 70 species of plasmodium species infective to different classes of vertebrates, ranging from monkeys (mammal) to lizards (reptiles) is known (Polozok 1989).

Man is said to be with several types of malarial parasites infectious in monkey, such as *Plasmodium knowlesis, P. cynomolgi bastianeli, P. Nui, P. simium* and *P. shorti*. They can be contracted both experimentally and under natural condition, with possible subsequent transmission to another man via mosquitoes. However, only four species of plasmodium are said to cause malaria in man. They include *P. falciparum, P. vivax, P. malariae* and *P. ovale*. But P. *falciparum* is the causative agent of tropical malaria. It is reported to have different strains; Indian, Italian, East – African and West – African etc. they differ in their sensitivity to anti malaria drugs and induced diseases with different severity. The peculiarity of the clinical course of falciparium malaria is largely attributed to the specific properties of the plasmodium, such as a sharply marked invasiveness, asynchronous development and latent sporulation (Polozok; 1989). In addition, the membrane of the parasitized erythrocytes develops processes and spike – like protrusions showing an antigenic affinity to the capillary endothelium. These processes form punctuate connection with the membrane of the capillary endothelium and with processes of other erythrocytes, which could be a direct cause of the retention of the parasitized red blood cells (pRBCs) in the organs. pRBC with *P. vivax* and *P. ovale* do not exhibit such changes (Polozok 1989).The causative agent of tertian malaria is *P. vivax*. It induces clinical infestation after a short

incubation period of (10 - 21 days) or a prolonged incubation of (6 - 14 months) period, these lead to a suggestion that, there are two subspecies of vivax malaria: *P.vivax* and *P. vivax_lubemans* (Polozok 1989). Another distinct feature is the appearance of "recurrence" after a long latent period. The theory of *P. vivax* sporozoite polymorphism was used in the explanation of the mechanism of such prolonged period of incubation and repeated long – term manifestations in vivax malaria. According to the theory different types of sporozoite of *P.vivax* are formed within the mosquitoe: tachy – spoprozoite (those capable of immediate development) and brandy sporozoite or hypnozoite (those that begins the cycle of tissue schizogony after a definite period of rest or dormancy).

P. ovale is similar to *P. malariae* morphologically and close to *P.vivax* in relation to its ability to induce changes in the pRBC. In blood smear *P.ovale* rings are similar to that of *P. vivax* and *P. malariae*. The ring occupies one third to half of the RBC, but have a large rounded or irregular nucleus. The cytoplasm looks like a thick rim narrowing to the nucleus. An erythrocyte may contain 2 – 4 rings.

The amoebic forms of schizonts have round shape without any marked pseudopodia and vacuole. Sometime it fills the whole RBC and contains pigment casts in form of short rods and lumps varying in colour from light to dark – brown.

Trophozoites are concave disk that appear as rings when stained. The center is thin and resists staining. The ring cytoplasm contains organelles and the nucleus is clearly visible as a single or sometime double chromatin dot(s). They feed on the hemoglobin and the cytoplasmic end product of RBC result to malarial pigment (seen as brown – black granule) (Polozok, 1989).

1.4 LIFE CYCLE OF MALARIAL PARASITE

The life cycle of malaria parasites spread over two different organisms: mosquitoes and human. It is a complex cycle of development with a change of hosts: The Sexual cycle of development (sporogony) in mosquitoes and an asexual cycle (schizogony) development in human.

Sporogony: Mosquitoes (Anophele) become infected with the gametocytes (sexual forms of the plasmodia) when it takes its blood meal from the malaria patients or carriers. In the stomach of the mosquitoe, one macro- gamete (from female gametocytes) and 4 to 8 exo-flagellated micro- gametes (from male gametocytes) are formed. Macrogamete is fertilized by microgamete to form zygote, which subsequently transforms into the motile ookinete. The ookinete penetrate through the stomach wall of the mosquitoes to the external membrane, where it becomes oocyst enshrouded in a membrane. The oocyst grows, replicating its content into large number (about 10,000) of sporozoite. The sporozoits are spindle like (11 – 15µm longs and 1µm wide).

The growth processes require a suitable temperature of about 16^0C(60F). Rapid replication is favoured as the temperature increases and ceases entirely below 16^0C (60F).

The incubation period associated with these processes is between 7 –10 days based on the species involved. Sporozoites are widely distributed through out the mosquitoe's body most especially the salivary glands. Such a mosquitoe becomes infectious and remains in that state for 1 – 2 months (Polozok, 1989; Cheesbrough, 1991 and Carrington 2001).

Schizogony: this is the asexual cycle of the organism. In human host plasmodia undergoes two asexual cycles of development. The hepatic cells exo-erythrocytic (tissue) schizogony cycle and red blood cells – erythrocytic schizogony cycle. The on set of the hepatic cell cycle is characterized by the invasion of the liver cells (hepatocytes) by malarial sporozoites injected by mosquitoe as it takes its blood meal from the human body.

The invasion is between 30 minutes to 1 hour after they are injected into the blood – stream. They grow, multiply and develop directly into schizonts in the hepatocytes. The schizonts mature and break open to release merozoites. This cycle is completed within 5 – 7 days and is asymptotic. Each schizont is about 60 μm and contains about 30,000 merozoites.

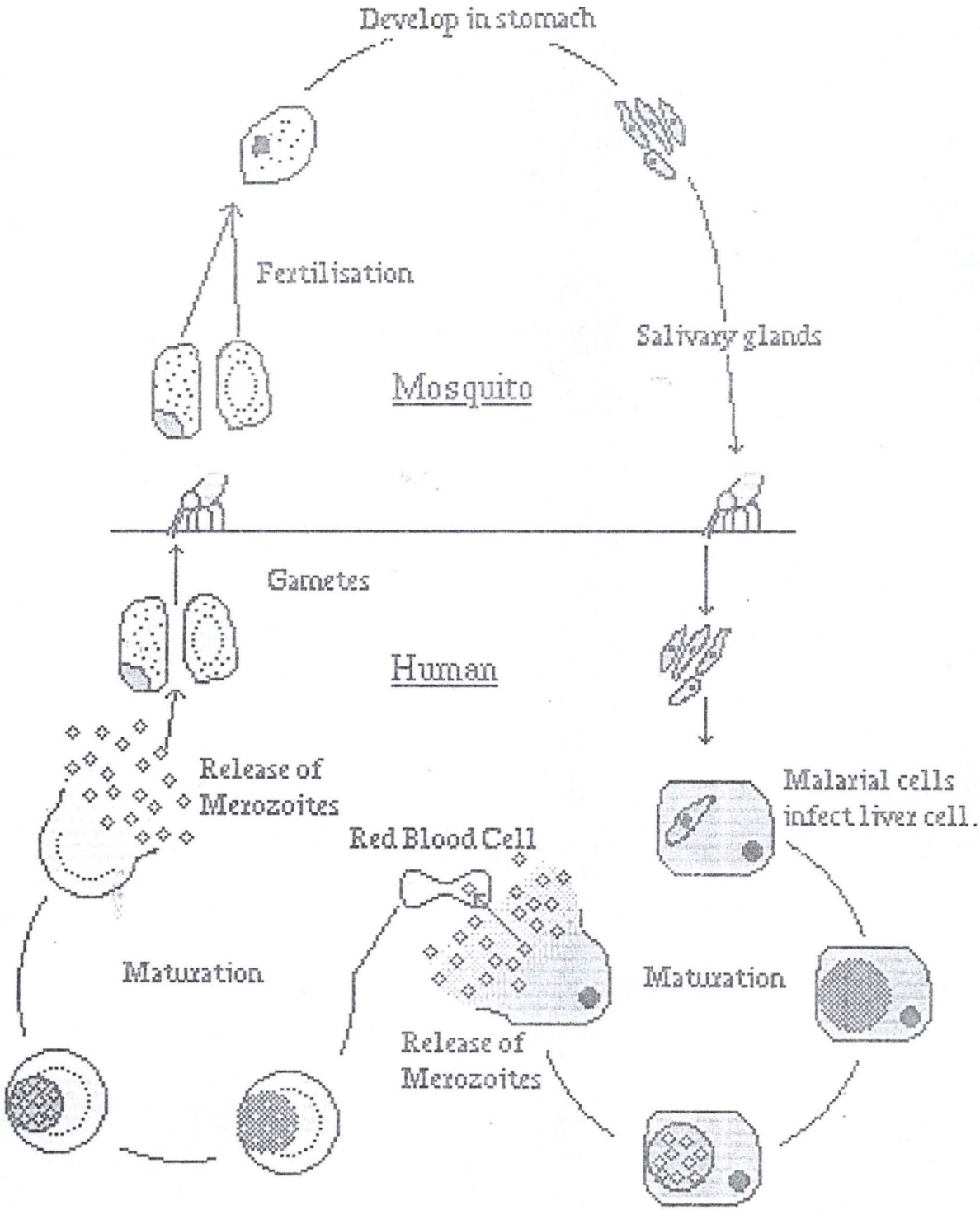
The merozoites released in the blood stream within a few minutes invade the erythrocytes for survival. This marks the beginning of the erythrocytic cycle. The penetration of merozoite is characterized by their interactions with the erythrocyte receptors and attaches to it by the apical part after which the erythrocyte membrane is deformed. The invagination takes place and the merozoites are drawn into the cell with the formation of the parasite form vacuole. Such process is possible only in the presence of the appropriate receptors on the erythrocyte surface. For *P. vivax,* such receptors are presented by Iso-antigens of blood group, Daffy (fy^a or fy^b) positive. Erythrocytes with Daffy negative (fy fy) do not have recognizable receptors and could not be penetrated by the parasites. Daffy – negative genotype occurs particularly, frequently in Negroes of West Africa (90 %) and in Negroes of North America (70 %), which accounts for their resistance to vivax malaria (Polozok 1989; Adams, 2001).

Glycophorines of the erythrocyte membrane appear to serve as receptors for *P. falciparum*. Absence of these receptors makes invagination of red blood cells impossible for the parasites. The parasites therefore died off and the cycle is terminated (Kreier 1980, Polozok 1989).

Following the invasion of the RBC, trophozoites are formed from merozoites and are embedded in a vacuole. The duration of this cycle is between 2 – 3 days. When they are fully developed, its nucleus begins to divide, followed by subsequent division of cytoplasm, which results in schizonts formation. It further ruptures to release about 8 – 32 merozoites (depending on the type of parasite), malarial pigments and toxins.

Some of the merozoites that are released perish and others invade another RBC for the cycle to continue. In the RBC some merozoites generate sexual forms i.e. male and female gametocytes in the ratio of 1:4. The gametes formed could survive without fertilization for only short time (few hours) in *P. vivax, P. ovale* and *P. malariae*, while that of *P. falciparum* extend to several weeks which accounts for its prolonged life cycle and contributed to its life threatening status (Polozok; 1989).

Figure 1.4- The life cycle of the malarial parasite, it is divided between humans and mosquitoes. The parasite develops in the stomachs of mosquitoes, and is transferred from human to mosquito and back when the mosquito feeds.



Adapted from; Malaria 2001. Disease type blood and Hearth.

Web-site: www.diseasedir.orh.uk/infect/bact.01.htm

1.5 TYPES OF MALARIA AND ITS MODE OF TRANSMISSION

There are four species of plasmodium, which cause malaria in human. They include *Plasmodium falciparum* which cause falciparum malaria called tropical malaria or subs tertian malaria or malignant tertian malaria.

Plasmodium vivax causes vivax malaria or tertian malaria. *Plasmodium ovale* on the other hand causes ovale malaria and *Plasmodium malariae* cause malariae malaria, which is also known, as quartan malaria. Each one of them has some etiological and epidemiological characteristics that distinguish it from the other.

However, of these species *Plasmodium falciparum* is the most significant, since it causes potentially fatal disease that has increased its world wide distribution and acquired considerable resistance to anti malarial drugs (Roberts and Campbell; 1997).

The peculiarity of the clinical course of this organism as it relates to malaria include:

i. Sharply marked invasiveness, i.e. capable of multiplying at an exceptional rate in just 1 – 2 cycles of erythrocyte schizogony, given rise to a very high level of parasitaemia. Example 5,000 cells per µl of the blood may grow to 100,000 cells and over, within 48 hours making it possible for 20 – 30 % of the RBCs to be invaded.

ii. Asynchronous development: This involves simultaneous presence of several fairly large generations of parasites, with different completion times of their developmental cycle in the blood. This is usually expressed in the overlapping of febrile illness.

iii. Latent sporulation i.e. the completion of schizogony occurs in the capillaries of the internal organs such as brain, spleen, liver, bone marrow and mucous membrane of the gastrointestinal tracts, unlike the other species that occur in the circulating blood.

P. vivax is the next most important numerically, not usually fatal but can remain latent for many months before becoming symptomatic. It could cause relapses over a period of many months (Town end, 2002)

The transmission of malarial parasites occurs in the appropriate climatic conditions and its intensity depends on the number of mosquitoes and the type of species they belong to. About 300 species and subspecies are known, which include: Mosquitoes of the genus *Anopheles gambiae* (account for most malaria infection in Africa), *A. atroparvus* (prevalent in England and USSR) and *A. culcifacie* (account for 60 – 70 % malarial transmission yearly in India). *A. sinensis, A. balabacensis, A. stephensi, A. fluviatilis, A. authropophagus, A. dirus, A. quadrimaculatus* and *A. albimanus* (in America) are also involved in malaria transmission in different parts of the world (Polozok; 1989; Tembeng; 1999).

The transmission is through the bite of the female anopheles mosquitoe during its blood meal, a pre–requisite for its egg development and production (Chris et al; 1997). Further report showed that malaria could be transmitted through blood transfusion from an infected person (Cheesbrough; 1991a). Intrauterine infection could occur through placenta or in the course of parturition when the blood of the mother and foetus mixed. If aseptic rules are violated, there may be cases of parenteral infestation via syringe and needles as well (Reed; 1997, Marsh; 1

1.6 IMMUNITY

One of the factors that influence the epidemiology of malaria is the immune response of the infected person. Acquiring natural immunity to malaria depends on repeated exposure to infection, which is not easily developed in an unstable transmission area (area where malaria transmission is seasonal or yearly) and slows even in stable transmission areas (area where transmission occurs for at least 6 months and is intense).

Immune mothers' Ig G antibodies and high concentration of haemoglobin F in the infant's red cells protects the infants in the first few months (about 3 month) of their birth (Cheesbrough, 1991a). At about 6 months, studies have shown that, infants' antibodies could not be demonstrated, following a drastic reduction in their resistance. This suggested that passive immunity diminish as the baby grows (Robinson, 2000). Following repeated infection an infant normally acquires a natural immunity to malaria at about the age of 5 years (Polozok; 1989).

This extends to more than a decade before it reaches substantial protection, which never reaches completion. However, the age at which maximum protection is reached correlate inversely with the intensity of parasite transmission (Robinson; 2000). The above observations could support the hypothesis that says the malaria antigens that induce protective immune responses might be very poorly immunogenic. In addition these antigens might be very polymorphic (varying between different strains of the parasite antigenic diversity) or variable (changing with time, within individual strains-antigenic variation). Hence the accumulation of immunological

memory to a large number of different antigenic epitomes is required before a substantial protection is achieved (Robinson, 2000). Recent findings suggested that an important way in which parasites can differ based on antigen, is in their expression of a particular parasite-encoded antigen called *Plasmodium falciparum*-infected Erythrocyte Membrane Protein1 (PFEMP1) as identified in *P. falciparuim* (Robinson, 2000). It is highly variable and is encoded by the diverse var-gene family. Approximately 50 different var-genes are present in each genome. PFEMP1 is of *P. falcipaum* parasites. It appears to play a central role in the adhesion of pRBCs to specific receptors in the host micro vasculature. Thus expressed on the surface of RBCs that are infected with the mature stages, it's critically important for the survival of parasites, since it prevents destruction of the infected RBCs during their passage through the spleen. So several studies point to its importance in protective immunity (Robinson; 2000).

Recent work by Bull et al, (1999), as quoted by Robinson 2000, on Kenyan children, showed that agglutinating antibodies that had specificity to several variants of the PFEMP1 receptor were found in a child ill with malaria, the antigenic variants of PFEMP1 that were expressed by parasites at the time of the malarial episode were often not recognized by specific antibodies in that child. This therefore suggested that it is the lack of recognition by antibodies that allowed sufficient multiplication of parasites to precipitate clinical disease. This suggests a protective role for antibodies against PFEMP1 in naturally acquired protective immunity (Robinson, 2000).

Some individuals living in malaria endemic areas have a natural or innate resistance to malaria. This is based on the inheritance of some selected resistance genes that are expressed on the red cells as a

result of mutations. Although such mutations can cause fairly serious problem for people in modern society, their high prevalence within an ethnic group suggested some advantages in the past, notably malarial resistance (Connor, 2001). Study by Tishokoff, (2001) showed that mutations in glucose-6-phosphate dehydrogenase (G6PD) genes almost certainly arose as a result of the emergence of malaria. The variation of the mutation was found to have appeared in several areas where the incidence of malaria was high. It was further noted that the mutation appears to have arose at about the same time that malaria became prevalent.

Some of the mutated genes that are selected apart from G6PD, includes: genes for Iso – antigens in Daffy blood group (fy^a or fy^b) (Polozok 1989; Adama 2001) that serve as parasite receptors, a factor contributing to its attachment to the erythrocyte, which account for the resistance in *P.vivax* malaria. Others include haemoglobin S genes; thalassaemia genes and ovalocytosis (Cheesbrough, 1991a) all confer resistance to falciparum malaria, a leading cause of morbidity and mortality. The mechanisms by which these gene modifications protect against malaria are yet to be fully elucidated.

However, cells containing Hb AS or SS do sickle more quickly when they contain parasites, probably because the parasites lower the pH of the erythrocytes, which leads to the damage and sickliness. Such damaged cells together with the parasites are phagocytosed (Cheesbrough, 1991a).

Thalassaemic cells are however, invaded by the parasites, but can be injured by the increased sensitivity of the cell membrane of such cells to peroxide damage (Cheesbrough, 1991a).

In ovalocytosis: (elliptical) red cells probably resist malaria due to their morphological abnormalities and antigenic compositions of their cell membranes. High incidence of ovalocytosis in falciparum malaria in endemic areas of Papua New Guinea has been reported (WHO, 1986). Several studies have provided evidences to link naturally acquired protection against *P. falciparum* in endemic population with immune response to particular parasite antigens. Examples of these includes:

- The levels of circulating antibodies to the merozoite surface protein1 (PFMSP1).
- The production of interferon gamma (IFN–g) response by T–lymphocytes (T – cells) in response to liver stage antigen 1(LSA– 1).
- Antibody – dependent cell – mediated inhibition (ADCI) – type responses to several parasite antigens, such as merozoite surface protein 3 (MSP – 3) and glutamate – rich protein (GLURP).

The relative importance of various types of immune responses to variable and conserved targets in the acquisition and maintenance of protective immunity is hotly debated and more studies in various endemic settings and populations are needed to resolve the apparent paradoxes and ambiguities (Robinson, 2000).

Recent study by Kurtis et al (1999) showed for the first time, that RU–bertal hormone levels are directly related to increased resistance to malaria in humans. While the mechanism remains unknown, the suggestion is that, this may be an acquired, rather than an innate form of immunity as exposure, subsequent to elevation of male sex hormones augments resistance in older, but not younger individuals.

The findings suggested that the protracted period needed to attain protective immunity could be explained as the consequence of human development, rather than as the requirement to recognize variant or poorly immunogenic parasite antigens. This raises the possibility that, an understanding of protective immune responses that are developmentally regulated could lead to novel vaccine strategies (Robinson, 2000.)

1.6.1 VACCINE DEVELOPMENT

Despite long and intensive research efforts, no vaccine against malaria in humans (which is both effective and suitable for mass production) is yet to be available. This could be explained on the bases that no model for effective immunity to plasmodia infection. Since incomplete immunity to malaria infection are featured in humans. The relative importance of the hormonal versus cell–mediated immunity is also unclear. In addition, both natural and vaccine induced immunity are hampered by the parasite capacity to vary its antigenic structures this result in different antigens of different stages of the parasitic infection (Karin and Peter, 2000, Robinson 2000).

For effective vaccine development, 3-different antigens that include pre–erythrocytic stage, the asexual blood stage and transmission (gamete) stage could be covered (Karin and Peter, 2000).

The current antigens in study as potential vaccines include:

- Circumsporozoite protein (CSP)
- Merozoite surface protein 1 (MSP – 1) (Karin and Peter, 2000 Robinson, 2000)
- Erythrocyte binding antigen 175 (EBA 175)
- Apical – Merozoite antigen (APA – 1)
- Gametocyte antigens (Pfs 25) (Karin and Peter, 2000)

1.7 SYMPTOMS AND CLINICAL MANIFESTATION

1.7.1 SYMPTOMS

In endemic areas most people who have malaria parasites in their blood may not be ill, others may develop mild malaria. A few will suffer severe malaria, which may be fatal if the treatment is delayed or not available (Molyneux, 1997). The characteristic feature of mild malaria is fever, which usually occurs in 3 stages.

i. **Cold stage:** Characterized by rigor headache, the patient feels cold and shivers though the temperature may be rising.

ii. **Fever stage:** Characterized with rises in temperature to the maximum, headache is severe, pain in the back and joint. A times its accompanied with vomiting and diarrhea.

iii. **Sweating stage:** This stage is characterized by perspiration of the patient. The body temperature falls and is relieved of headache and pains.

Moreover fever may have occurred on alternate days, more commonly continuous or irregular in some cases, particularly fever caused by *P. falciparum* (Cheesbrough, 1991a, Molyneux 1997).

Severe malaria (complicated malaria) is considered severe when more than 5% of the red blood cells become parasitized. This occurs most likely in a non–immune person or person with lapsed immunity. Pregnant women particularly the primigravidae (first pregnancy) are mostly at risk, followed by gravidae and multigravidae (Singh; 1999).

Singh (1999) reported that the prevalence of *P. falciparum* malaria among pregnant women was highest early in the second trimester and 3rd trimester for *P. vivax*. This observation has often been interpreted as a consequence of the immuno-suppression that is

necessary to protect the foetus from being rejected by the mother's immune system (Robinson, 2000). This however, could not give enough explanation to the higher susceptibility recorded in primigravidae. The contribution of chondrotin sulfate A, (CSA) a ligand that is present in the placenta syncyto trophoblast only and not readily accessible on cells else where in the body, is also attributed to higher susceptibility in the primigravidae (Robinson, 2000). Parasitized red blood cell (pRBC) preferentially binds CSA with the mediation of a variant of PFEMP1. Any parasite with adhesion specificity for CSA are eliminated from non-pregnant individual owing to lack of suitable adhesion receptors in the host cells and presumably before they have induced appreciable level of antibodies to the CSA–specific PFEMP1 variant (Robinson, 2000). However, because of the presence of CSA in the developing placenta of primigravidae, parasites that are able to bind CSA present in the blood can multiply unhindered. But with successive pregnancies the levels of the antibodies directed against PFEMP1 variant molecules that bind CSA increase and limit the multiplication of CSA – binding parasites (Robinson, 2000).

Other people susceptible to severe malaria include, children (Luxemburger, 1997), immuno-suppressed person following splenectomy (Polozok, 1989). Several cases are mostly associated with *P. falciparum* malaria or mixed infection of *P. falciparum* with *P. vivax* or others (Reed, 1997).

Certain complications associated with *P. falciparum* malaria include: cerebral malaria, most commonly in children and pregnant women (Luxemberger,1997; RBM,1999; Abuja, 2000). Cerebral malaria results when RBC and fibrins block the blood capillaries and venules in the brain. It is also characterized with neutrophillia, cerebrospinal fluid (CSF) may contain malaria parasite cells. It includes rise in

proteinuria, hypoglycemia and increase in CSF lactate level (Luxembuger, 1997;).

The possible effect of cerebral malaria includes, confusion, coma and convulsion with high mortality rate. (Luxembuger, 1997). Studies have shown high level of cytokine tumor necrosis factor alpha (TNF-a) in fatal cases of cerebral malaria (Robinson, 2000).

Anaemia is one of the complications associated with *P. falciparum*. It is a major problem among children and pregnant women. (Reed, 1997). It is said to account for low birth weight, miscarriage, premature labour and stillbirth in pregnant women (Reed, 1997:). Anaemia in malaria is due to mechanical destruction of parasitized red blood cells. Bone marrow red cell-production is reduced. (Polozok, 1989; Reed, 1997). An auto immune destruction of red blood cells may occur, which could bring about black water fever, an acute condition characterized by rapid and massive intravascular haemolysis of both parasitized and non-parasitized red cells. This result in haemoglobinemia, and haemoglobinuria, which is also accompanied by high fever, vomiting, jaundice and often sudden death. The urine appears dark red to brown black (thus the name: black water fever) due to the presence of haemoglobin in form of metha haemoglobin and oxyhaemoglobin (Polozok, 1989;). The urine contains protein, hyaline and granular casts and epithelia debris (Heinemann; 1972).

Malarial pathogenicity is associated with damage brought about on some special organs, such as the brain, liver and kidney. The most pathogenic of the human malaria species is *P. falciiparum*. Its pathogenicity is mainly due to;

a. Erythrocyte schizogony taking place in the deep capillaries of organs such as the brain, heart, spleen, intestine, lungs, bone marrow and placenta (Townend; 2002).

b. Changes on the surface of parasitized red cells, which brings about marked deformity (as a result of reduction in pH of RBC) caused by the adhesion of the red cells to each other and to the cells lining the walls of capillaries. This result in sequestration of infected red cells in the capillaries of internal organs. Which brings about congestion, hypoxia, blockage, decreased oxygen transport and increased fragility, with subsequent rupture of the small blood vessels, toxin production and antigen released (Townend, 2002; Tembeng, 1997).

c. Level of parasitaemia the introduction of "the pyrogenic threshold", i.e. the level of parasitaemia that has to be reached before the attacks of malaria begins (100 parasites per µl of blood in *P.vivax*, 600 parasite per µl in *P. falciparum*) is also an important factor in the pathogenesis of malaria. However, *P. falciparum* is distinct in this case with higher levels of paracetaemia per µl of blood, i.e. about 10–40 % RBCS become infected (Heinemann, 1972). The pyretic reaction from the physiological viewpoint, are due to the responses of the hypothalamic thermo regulation centres to the effect of various pyrogenic irritants, which are suggested to be toxic metabolites of the parasites (Polozok, 1989).

1.8 MALARIA AND KIDNEY DYSFUNCTION

The association between parasitic infection and kidney disease has been recognized since antiquity long before the discovery of the causative organism (Hutt, 1979). The first parasitic infection that was clearly shown to be associated with kidney in tropical area was malaria. (Prant et al,1974; Van Velthysen; 1996). The earliest investigators associated it to quartan malaria and designated it as "quartan malarial nephropathy (Hendrickse et al, 1972;) or nephrotic syndrome (Rees et al, 1972; Herbert, 1972; White, 1973). It occurs with considerable frequency in the tropics where malaria is prevalence especially in children (about 60 to 90 times greater) in malarious area than non – malarious area (Houba, 1979). After several years of pain staking observation, substantial evidence of a link between quartan malaria and nephritis in British Gurana was reported (Voller et al; 1971, Glasgow et al; 1972, White, 1973). In Uganda quartan malaria has been implicated as an important aetiological factor in the nephrotic syndrome, not only in children, but also in adult [Rees et al; 1972; Abdulrahman et al; 1983a) reported nephritic-syndrome to be characterized by high *P. Malaria* paracetaemia and elevated serum *P. Malariae* antibody. Persistent heavy proteinuria, hypo-albuminaemia <2.0gldl and generalised oedema (Hendrikse et al; 1972; White, 1973).

Normally only a small amount of protein crosses the glomerular capillary wall, which is reabsorbed in the proximal tubules. When the permeability of the glomerular capillary wall is increased by glomerular inflammation changes in surface charge and alteration in pore size. The amount of protein presented to the tubules therefore exceeds their reabsorptive capacity and protein spills over into the

urine [Tilkian, 1979; Edward and Bouchier; 1991]. Nephrosis was earlier reported to be unique among malarial complication characteristic of quartan malaria (Herbert, 1972; Prakash et al 1992] however, reported *P. falciparum* as also an important factor in the aetiology of acute renal failure in part of India. The syndrome was said to be transient and characterized by raised blood urea, low urine specific gravity and low ratio of urinary to blood urea (Mukherjee et al,1971; Van – Velthvysen, 1996).

The glomerulonephritis is immunologically often thought to be mediated this is because of the glomerular changes seen with immunoglobulin deposition. It was suggested that the corpuscular parasitic antigen released from the erythrocytes processed and changed into another soluble form which either deposit in the tissue and then react with the antibodies (Local formation of immune complex) or enters the circulation and bind with antibodies into soluble immune complexes and localized in the vessels (Houba, 1979).

It was further suggested that DNA – binding antibodies and cell – mediated immune mechanisms play a role in the development of the nephritis during murine malaria (Rui – mei et al, 1998b). Murine model studies demonstrated strong correlation between major histo-compatibility complex (MHC) class1 expression in the glomeruli; MHC Class II expression in the glomeruli / proximal tubules; and immune cells positive for CD4+ and CD8a+ infiltrates in the tubulo interstitial with severity of renal dysfunction (Rui – mei et al 1998c).

In a related finding Sinnah et al (1999) reported a strong correlation between the expression of tumour necrosis factor-alpha (TNF–alpha) with inter-teukin–6 (IL–6) and inter-leukin–1 alpha (IL–1 alpha) with IL–6.

He further reported that the expression of TNF–alpha, IL–1 alpha, IL–6 and IL–10 was found to be strongly correlated with severity of proteinuria. And thus showed that an up–regulation of cytokines in the pathogenesis of glomerulo–nephritis is associated with murine malaria infection. The development of glomerulo–nephritis progress to renal failure in malaria falciparum if left untreated or wrongly diagnosed (Mishra et al 2002).

Acute renal failure in malaria was said to be mediated through several mechanisms, which may be as a result of the effect of parasitized (RBC) on the micro–circulation, hypovolaemic shock or non–specific effects of inflammation (Prakash, 1996; Mishra et al; 2002). These effects could be explained in the following terms:

a. Effect of RBC on the micro – circulation; Parasite penetration of RBC produces changes on the surface of the pRBC, causing the formation of knob like processes, which helps in anchoring the endothelium and adhesion between the RBCS. These tight packed the RBCS and impede the micro–circulation to the vital organs. Cyto-adherence due to thrombospodin formation from vascular endothelium (Peculiar to *P. falciparum*.) RBCS inability to deform according to the need of micro – circulation leads to sluggish blood flow and consequently to renal ischiaemia.

b. Hypovolaemia (low blood volume); may occur due to fever, (hyper–pyrexia) sweating or decreased intake of fluid.

c. Non–specific effects of inflammation: there may be leakage of fluid from intravenous compartment due to increased vascular permeability.

d. Intra – vascular coagulation

e. Increased plasma viscosity due to infection

f. Release of chemical mediators (TNF etc), which lead to vaso-constriction effect, increases cate--cholamine release and increases vascular permeability.

g. Hyper--bilirubinaemia: a high level of bilirubin has a contributory effect in the pathogenesis of acute renal failure. This is as a result of haemolysis, which causes alteration in renal haemodynamics. In addition, it brings about depression in cardiac function, which could result in severe jaundice, that induces hyper--uricaemia. The end result of which is compromise in renal function in the presence of decreased acid urine flow.

Black water fever is occasionally associated with acute renal failure caused by Glucose--6--phosphate dehydrogenase deficiency.

h. Bacterial endotoxaemia may potentiate ischaemic renal injury (cytokinins, Cachectines and TINF etc). Cachectin can cause haemo-concentration, shock and tubular necrosis (Prakash, 1996; Mishra et al; 2002).

1.9 PREVENTIONS AND CONTROL OF MALARIA

Multi–dimensional approaches are used to effectively prevent and control malaria. These include:

i. Vector control
ii. Using drugs
iii. Education and training program
iv. Introduction of vaccine

1.9.1 Vector Control: - Individuals could avoid being bitten by mosquitoes, since malaria illness cannot occur without it. Hence it is of prime importance to avoid bites. However this could not be relied upon completely. Therefore, there is need for the use of both drugs and avoidance of bite even though no drug prophylaxis is 100% effective (Town end, 1999). The following could effectively control vectors:

a. Insecticide treated bed net (ITBN); the bed nets are impregnated with chemicals such as permethrin.
b. Insecticide spraying such as DDT on the breeding site of the vector.
c. Exploration of the use of the indigenous natural plant species and artificial mosquitoe repellants. Preparations, such as diethyltoluamide (DEET), lemon eucalyptus base preparation (mosiquard) and a new compound KBR 3023 (Bay repel) which appears to be both safe and effective could be used effectively to repel mosquitoes (Townend ;1999). It also includes oil of citronella, dimethyl phthalate and smoke from fire or from burning pyrethrum pellets (Cheesbrough, 1991a).
d. White wash of the walls of mud huts in rural area in particular to avoid attracting mosquitoes. Cracks and Crevices where stagnant water collect should be sealed (Carrington, 2001).

e. Selecting healthy sites for houses on the tops of hills, avoiding mosquito-breeding sites when ever possible. Screening windows and doors with mosquitoe netting.

f. Wearing protective cloths such as long trousers, long skirts, sarongs and garments with long sleeves (Cheesbrough, 1991a)

1.9.2 TREATMENT OF MALARIA BY THE USE OF DRUG

This treatment could be supportive measures as well as specific anti–malaria drugs. It is important that patients with *P. falciparum* malaria have their treatment commenced without delay, and they should investigate for any evidence of complications.

Severe malaria should be managed in an intensive care unit where close monitoring, fluid resuscitation and electrolyte balance can be achieved (Karin and Peter, 2000; Mishra et al, 2002). Acetaminophen should be administered for fever, benzodiazepines for seizures, glucose supplementation for hypoglycemia. Early dialysis for acute renal failure should be commenced and positive pressure ventilation for non–cardiogenic pulmonary oedema should be instituted (Karin and Peter, 2000).

Anti malarial drugs Such as quinine, chloroquine and artemisinin are the main stay of therapy (Mishra et al, 2002). Chloroquine is the most widely used anti malarial in the world. It is highly effective against clinical attacks of *P. vivax, P. ovale* and *P. malariae* malaria and sensitive to infections of *P. falciparum* malaria (Trigg and Rietveid, 1997).

It is important to note that no anti malarial drug currently availiable acts on all stages of the malaria life cycle to stop or kill the parasite.

However, drugs available for treatment of malaria and their mechanisms of action include the following:

- Quinoline derivatives: Chloroquine, quinine, quinidine, amodiaquine, mefloquine, halofantrine and primaquine. All inhibits heme polymerase activity. It results in free heme accumulation, which is toxic to the parasites. Chloroquine also inhibits the release and action of TNF–alpha.

  The other six drugs kill parasites in the intra–erythrocyte phase. Primaquine kills both intra-hepatic forms of the parasite as well as gametocytes.

- Anti-folate: Pyrimethamine, Sulfonamides, dapsone and proquanil. Fansidar is a combination of pyrimethamine (25mg) and sulfadoxine (500mg). These drugs accomplish there anti parasite activity by inhibition of folate metabolism, resulting in the inhibition of nucleic acid synthesis (Dipalma 1971). The drugs kill intra hepatic forms of the parasite, but not hypnozoites. Pyrimethamine also kills the sexual forms (gametocytes), which prevents transmission to the mosquitoe (Karin and Peter, 2000).

- Artemisinin derivatives–Includes: artemisinin artemether and artesunate. They bind iron in the malarial pigment to produce free radicals that damage parasite proteins.

- Anti–microbial: Clindamycin, atovaquone and tetracycline. They act synergistically with quinoline derivatives to kill blood schizonts.

- Resistance to anti malaria drugs: Chloroquine resistances have been observed in isolated strain of *P. vivax* in Africa. The mechanism of

resistance is not known, but is probably mediated through reduced uptake or rapid excretion of the drug.

The resistance to chloroquine in *P. falciparum* malaria is now widely spread in all countries. It is said to be due to an efflux mechanism in which the drug is pumped out of the cells by the parasites. The genetic basis that brings about the mechanism is yet to be defined.

Primaquine, quinine, mefloquine and halofantrine resistance has been reported in different places. However, the resistance is said to be clinically insignificant, hence their uses in combination remains highly efficacious (Karin and Peter, 2000).

The resistance of *P. falciparum* to mefloquine and halofantrine is associated with amplification of Mdr–life genes (Karin and Peter; 2000). Antifolate resistance in *P. falicparum* is prevalent in Africa. The resistance occurs via point mutations in the parasite dihydro folate reductase–thymidylate syntheses enzyme, this lead to decrease in drug binding at the active site (Karin and Peter; 2000).

Resistance could develop rapidly to anti microbial, such as clindamycin and atovaquone, as they are used as monotherapy against *P. falciparum* infections.

Resistance to atovaquone is mediated by single point mutations in the cytochrome–b gene. Most *P. falciparum* remain sensitive to tetracyclines. No resistance to the artemisinin derivatives has been reported (Karin and Peter; 2000).

Due to the above mechanisms of action and drug resistance, the following recommendations are made for treatment regimens as in the table below:

TABLE 1

TREATMENT OF MALARIA

	Oral Regimen	Parenteral Regimen
Chloroquine Sensitive *P. vivax*, P. ovale, P. malariae or *P. falciparum*	Chloroquine 10 mg base /kg (max 600 mg) then 5 mg base/kg (max 300mg) at 6, 24 and 48 hours	Chloroquine 10 mg base/ kg (max 600 mg) over 8 hours than 15 mg base/kg (max 900 mg) 24 hours (close monitoring).
Chloroquine resistance *P. falciparum*	Ouinine sulfate 10 mg salt /kg 8 hourly 3 – 7 days. plus Pyremethamine – sulfadoxine (25/500 mg) fansidar 3 tablets on day 3 OR Doxycycline 100 mg B.D or Tetracycline 250 mg O.D 7 days OR Mefloquine 15mg base/kg (max 750 mg) then 10 mg 1kg (max 500 mg) at 6 -8 hour in 1 week. OR Halofantrine 8 mg / kg	Ouindine glucenate 10 mg base / kg (max 600 mg) over 2 hours then 0.002 mg base/kg per minute (EKG monitor in ICU). OR Ouinine dihydrocloride 20 mg salt/kg over 4 hours then 10 mg salt/kg over 2 – 8 hours (max 1800 – mg / day) EKG monitor in ICU)

Ouinine resistance *P. falciparum* Prevention of relapse of *P. vivax* and *P. ovale*	(max 500 mg) O.D 6 hourly 3 close, repeat in 1 week Artesunate 4mg/kg Q/D for 3 days plus mefloquine (as above) OR Doxycycline (as above) Primaquine 0.2 mg base/ kg (usual dose 5 mg) Q/D for 14 days.	Artemether 2.4 mg/ kg than 1.2 mg/kg at 12 and 24 hours, then 12 mg/kg Q/D. OR Artemether 3. 2 mg / kg 1M then 1.6 mg / kg Q/D 1M. OR Ouinine sulfate (as above) plus. Doxycycline 100 mg B/D

Adapted from Stanley, J. Malaria Emergency med clinic in N. America 1997, 15:113 (Karin and Peter; 2000).

If patients cannot however tolerate oral therapy or have a parasitaemia of greater than 5 %, they should begin therapy with intravenous continues infusion as indicated in the table above. Other treatment include:

- **Exchange Transfusion:** - This is considered for severe complicated malaria due to *P. falciparum*, particularly if the parasitaemia is greater than 10% or there are life–threatening

complications. The transfusion rapidly removes parasitized red cells and parasitic toxins and replaces them with fresh plasma and unparasitized erythrocytes. It should be combined with drug therapy and should be continued until the level of parasitaemia is < 5%.

- **Desferrioxamine:-** is an iron chelator and is reported to have beneficial effects. Free iron form the heme in malaria pigment catalyzed a reaction that produces super oxide and oxygen radicals, which lead to tissue damage. Desferrioxamine binds free iron thus reducing tissue damage. It is also reported to have anti parasitic effect in–vitro and enhances parasite clearance in – vivo (Karin and Peter; 2000).
- Considering the devastation caused by malaria (such as high mortality and morbidity rate across the families particularly children and pregnant women) worldwide and its endemic in African (Nigeria in particular). Sustainable control measure is highly required. However, due to poverty, grossly in adequate health services and reports of drug resistance malaria remains a life threatening in Nigeria. Therefore it is essential that adequate protection by available means should be put in force. In addition, health education should be provided to increase the level of awareness of the potential risk, especially malaria complication which renal dysfunction is one.

Nephritic syndrome and glumerulo–nephritis are the characteristic feature of this condition as documented earlier (Abdurahman et al, 1983, and Ogbadoyi and Tembeng, 1999). Reports obtainable in this regard were centered on Zaria (Northern Nigeria).

This research work is designed to make further finding on the association and peculiarity of malaria and kidney dysfunction in population studied in Minna.

CHAPTER TWO

MATERIALS AND METHODS

2.1 MATERIALS

- **REAGENTS / CHEMICALS**

The reagents and chemicals that were used in this research work were obtained from General Diagnostic, division of Warner Lambart Company Morris, Mains, New Jersey USA; Laborature Biotrol 74140 Paris France, BDH chemical Ltd, Pool England and SIGMA chemicals Co. Ltd Pool England. All reagents and chemical used were of analytical grade.

- **SAMPLES**

- **Urine Samples**

Early morning urine samples were collected from patients, visiting the clinic of the Federal University of Technology Minna. The doctor diagnosed these patients to have malaria. They showed clinical manifestation of malaria and were confirmed to have malaria by the detection of malaria parasites in blood smears.

Samples were also collected from apparently healthy individual to serve as controls. All samples were collected in sterile sample bottles and taken to the laboratory for analysis.

- **Serum samples**

Using sterile syringes and needles, blood sample were collected from malaria patients and also from apparently healthy individuals. Blood samples were centrifuged, the serum collected, kept in sterile vials and stored frozen until required for analysis.

2.2 METHODS

2.2.1 Estimation of protein in urine

- **Preparation of standard protein calibration curve.**

  0.1ml of 30% commercial bovine serum albumin (BSA) was accurately measured and de ionized water added to make up to 30ml (forming a concentration of 1 mg / ml).

  This served as the stock solution of BSA and was stored in the refrigerator. From the stock solution different concentrations (0.2, 0.4, 0.5, 0.6, 0.8 and 1.0mg / ml) were prepared and protein concentration estimated by Biuret method (Singh, 1990).

The spectrophotometer (spectronic 20D +, Miltan Ray,) was switched on and allowed to warm up for about 10 minutes. The machine was set at zero with blank and the absorbances of samples were measured at 540 nm. The absorbance obtained was plotted (vertical axis) against concentration of protein in mg/ml (horizontal axis). A linear calibration curve was obtained (Appendix1c)

Estimation of the protein in urine using Biuret method The Biuret method of Singh (1990) was used with slight modification. Briefly, 5ml of cold 5% Trichloro acetic acid was added to 1ml of urine in cold. After 20 minute on ice, the preparation was collected and centrifuged at 3000 rpm for 10 minutes. The protein precipitate was dissolved in 1.5 ml 10 % NaOH (instead of 6 ml 10 % NaOH as indicated by Singh 1990).

1ml of this preparation was added to 6 ml of Biuret reagent and 0.5 ml of deionized water was added. After about 30 minutes, the spectrophotometer was set at zero with the blank and the absorbance was measured at 540 nm.The concentration of the protein was obtained by reading the values from the calibration curve provided (Appendix 1c).

2.2.2 ESTIMATION OF SERUM UREA

The diacetyl monoxine method (Cheesbrough, 1991b) was used

PROCEDURE: - 0.1ml of the serum samples were dispensed into boiling test tubes and 1ml of distilled water added (1:10 dilution) 1ml each of acid (H_2SO_4 and H_3PO_4) and urea colour reagent were then added. The test tubes were set to include standard (10µl urea standard) and blank. Urea reagent was added to the standard and blank test tubes in equal amount as in sample.

The preparations were shaken vigorously until they foamed and incubated in boiling water, at 100°C for 20 minutes for colour development. The test tubes were removed and cooled for about 5 minutes. The readings were taken using colorimeter, screen master (Hospitex Diagnostic Firenze, Italy) at wavelength of 505 nm. The machine (screen master) gives the concentration reading in mmol/L.

2.2.3 DETERMINATION OF SERUM CREATININE

Alkaline picrate–slot method(Cheesbrough, 1991b) was used.

PROCEDURE: - Four or more test tubes were set up in a rack (depending on the number of tests) to include the following:

B - Blank (containing 1ml creatinine working solution only).

S - 0.2 ml creatinine standard (88.8µmol/L.)

T - Serum samples (test 1 - 10 each time.) 0.2 ml of each sample.

1 ml of the prepared creatinine-working reagent was pipette in to each tube mixed well and left to stand at room temperature for 10 minutes.

The concentration was measured at a wavelength of 505 nm using Colorimeter (screen master).

2.3 ELECTROLYTE DETERMINATION

2.3.1 DETERMINATION OF SERUM SODIUM AND POTASSIUM IONS (Na^+ and K^+).

Flame photometry method (Davidson and Henry,1979).

PROCEDURE: some universal beakers were set up which include,

Blank - contains ionized water.

Standard - contains sodium standard; 0.I ml of 200 mmol/L. sodium chloride (NaCl) and Potassium standard; 0.1ml of 10 mol/L. potassium chloride (KCl) and 20 ml of de-ionized water,

Test sample - 0.1ml of serum and 10 ml of de- ionized water are added; given a dilution of 1:100 in both standard and sample beakers.

SODIUM: the filter pointer was turned to indicate sodium light and the galvanometer was switched on. The gas supply was fully on and the flame ignited, while air supply was turned on. The air regulated to about 0.9 lb/square inch (of meter indicating the level of gas). The gas knob was then adjusted so that discrete cone of flame was obtained. The galvanometer scale was set to zero with de-ionized water (Blank), followed by setting the photometer reading to 50 unit of the galvanometer scale, using the working standard. The machine was then reset to zero again. The test samples are then read, while checking the standard after 2-3 test samples are read.

POTASSIUM: The filter knob was turned to indicate potassium light. The galvanometer was then set with potassium working standard to 70 units and the procedure continued as for sodium.

CALCULATION: The amount of Na^+ or K^+ is given by the expression. mmol/L. = Galvanometer reading x factor,

The factor for Na^+ is 2 and for K^+ is 0.1

It is important to note that for monovalent cat ion mEq/L = mmol/L.

2.3.3 DETERMINATION OF SERUM BICARBONATE (HCO_3^-) TITRIMETRIC METHOD

PROCEDURE: 0.1ml (100 µl) of standard and test samples were dispensed into separate beakers. To each beaker 2.5 mls of deionized water and 1ml of 0.1N H_2SO_4 were added then swirled to mix well and expel the carbon (iv) oxide therein. One drop (0.1ml) of phenol red was added to each of the beakers.

Micropipette was then filled with 0.1N NaOH solution and used to titrate against the contents of the beakers until the colour changed to pink and is stable for about 15 seconds. The colour is matched with that of the standard.

CALCULATION: The amount of bicarbonate is given by the expression.

mmol/L= A x 100 where

A = 1 - volume of NaOH used (pipette reading)

2.3.4 DETERMINATION OF SERUM CHLORIDE BY TITRATION METHOD

PROCEDURE: 0.l ml (100 µL) of test serum sample was dispensed in to a universal beaker and 1.0ml de-ionized water was added (1:10 dilution). A few drop of biphenyl–carbazone was added as an indicator. The preparation was titrated, with the mercuric nitrate solution using a micropipette, until an endpoints was reached (Violet – blue color noted). The titration was carried out on 1.0ml standard chloride.

$$\text{Chloride (mmol/L)} = \frac{\text{Titre for test}}{\text{Titre for standard}} \times 100$$

2.4 STUDY POPULATION

The patients showed clinical manifestations of malaria and were confirmed to have malaria by the detection of malaria parasites in blood smears prepared from samples obtained from patients. A total of 146 males were considered for the work; 43 served as control group, 18 were children and 85 were adults. A total of 114 females; 15 as the control group, 17 children, 49 non-pregnant adult women and 33 were pregnant women. All the categories involved were tested for proteinuria, serum urea, creatinine and electrolytes (Na^+, K^+, HCO_3^- and Cl^-), using the routine procedures for the tests obtainable in general hospital Minna. The tests for Proteinuria were carried out in Biochemistry laboratory, Federal university of technology, Minna.

CHAPTER THREE

RESULTS

3.1: ANALYSIS OF PROTEIN CONCENTRATION IN URINE OF MALARIA PATIENTS AND HEALTHY INDIVIDUALS.

3.1.1: Descriptive analysis shows that out of 18 male children, 13(72.2%) have marked proteinuria with mean value of 24.79± 4.06. While 58(68.2%) out of 85 male adults have protein level in urine above the normal value with mean value of 23.67 ± 1.64. This varies significantly with mean value of the healthy individuals 12.00± 0.72(Table3.1a). 14(82.4%) of the17 female children malaria patients have marked proteinuria above normal value; with mean value of 33.87± 6.48. 30 (61.2%) of the 49 adult non pregnant female patients have protein concentration in urine above normal with mean value of 23.2± 2.19, 21(63.6%) of 33 pregnant women patients have high level of protein with mean value of 24.62± 2.13. These mean value varies significantly with the mean value of 14.68± 1.60 of the healthy individuals (Table 3.1a).

3.1.2 :One-Way analysis of variance indicated that the mean value for the protein concentration in urine of male healthy individuals, children and adults are 12.00 ± 0.72^{a} 24.79 ± 4.06^{b} and 23.67 ± 1.64^{b} respectively (Table3.2a). While female patient children, adult non-pregnant and pregnant women are 33.87 ± 6.48^{b}, 2.81 ± 2.19 ^{ab}and 24.62 ± 2.13^{ab} respectively. The healthy individual have 14.8 ± 1.60^{a} (Table 3.2b) variation in the superscript indicated ($P< 0.05$) significant variation.

3.1.3: Correlation matrix (Coefficient) for the male patients shows that protein is positively correlated with MPS with the value of

0.32*(Table3.3a), and in female malaria patients groups, with value of 0.23* (Table 3.3b).

3.1.4: Regression analysis (model fitting) show that protein concentration in urine is directly related to the MPS (level of infestation) as indicate by 0.02* and 0.01* for both males and females respectively (Table 3.4).

3.2: ESTIMATION OF SERUM UREA IN MALARIA PATIENTS AND HEALTHY INDIVIDUALS.

3.2.1: Descriptive analysis of the serum urea indicated that the mean value of the healthy individuals for both males and females are 4.96± 0.22 and 4.91± 0.05 respectively. 5(27.8%) of the male children patients have hyperuremia, while the female children patients have 6(35.3%) out of 17. The mean values are 4.39± 0.56 and 5.54± 0.79 respectively. Male adult patients have 16 (18.8%) out of 85 with mean value of 5.26± 0.35 while in female non pregnant group, only 4(82%) out of 49 have high level of serum urea ,with mean value 3.99±0.26. pregnant women patients on the other hand, 3(9.1%) out of 33 have high level of urea above normal range and mean value 3.57± 0.27 (table 3.1a).

3.2.2: One-way analysis of variance revealed that urea level in male malaria patient groups is not significant since they bear similar superscript (Table 3.2a). ($P < 0.05$) significant variation is indicated with the female groups and the healthy individuals with mean values 4.91 ± 0.55^{ab} , 5.54 ± 0.79^{b}, 3.99 ± 0.26^{a} and 3.57 ± 0.27^{a} for healthy individuals, children non-pregnant women and pregnant women respectively (Table 3.2b). While significant variation is indicated between the adult patients and healthy individual no significant variation exist within the 2 groups of

females (i.e. pregnant and non pregnant women) since they bear same superscript (Table 3.2b)

3.2.3: Correlation coefficient shows that urea is negatively correlated with MPS in female patient groups with value -0.21*.(Table 3.3b) and no correlation is indicated in male groups (Table3.3a).

3.2.4: Regression analysis indicated no relationship between urea and MPS (level of infestation) in both male and female groups (Table 3.4)

3.3: ANALYSIS OF SERUM CREATININE IN MALARIA PATIENTS HEALTHY INDIVIDUALS

3.3.1: Descriptive analysis shows that 4(22.2%) of the 18 male children have elevated creatinine level with mean value of 103.44± 7.21, while 6 (35.3%) of the female children patients have values of creatinine higher than normal, the mean value is 119.59± 1.78 (Table 3.1a). 22(25.1%) of 85 male adults patients have high creatinine level in serum with the mean value of 115.76± 4.01, while adult female (both pregnant and non pregnant) have 10.2% and 15.2% of their total number of case analyzed to have serum creatinine above normal with the mean values of 99.03± 4.20 and 102.37± 4.45 respectively.

3.3.2 One-Way analysis of variance shows that creatinine level of the healthy individual and patient groups are statistically insignificant since the mean values bear similar superscript (Table 3.2a and 3.2b)

3.3.3: Correlation matrix indicated correlation between creatinine and urea in male patients with value 0.85* (Table 3.3a) and in female patients with value 0.78* (Table 3.3b)

.3.3.4: Regression analyses show no relationship between creatinine level and MPS In all groups of patients analysed. (Table 3.4)

3.4: ANALYSIS OF SERUM SODIUM (NA^+) IN MALARIA PATIENTS AND HEALTHY INDIVIDUALS

3.4.1: Descriptive analysis of result show that the mean values of the serum sodium for healthy individuals for males and females are 129.5± 1.01 and 126.73±1.89 respectively. While 1 out of 18 male children, (5.6%) has the sodium level above the normal, 11(61.1%) have their serum sodium level lower than the normal range

(Table 3.1b). In the female groups 8 (47.1%) of the female children patients have their serum sodium level above normal (Table 3.1b) .52 (61.2%) of the male adults group have their serum sodium below normal (Table 3.1b), while 1 (2.0%) of the female non pregnant women has her sodium value above normal. 24(49%) have their own below normal. Pregnant women on the other hand have 16 (48.5%) cases to have sodium level below the normal value (Table 3.1b).

3.4.2: One-Way analysis of variance for serum sodium indicated that the mean values of the healthy individuals, children and adults for males are 129.51 ± 1.01^a, 131.61 ± 2.00^a and 130.72 ± 0.84^a respectively and the values bear the same superscript and are therefore insignificant. However, female patient groups with mean values 126.73 ± 1.89^a, 133.65 ± 1.34^b, 133.08 ± 1.06^b and 133.64 ± 1.04^b show significant difference with the healthy individuals because of the difference of superscript the values bear. But no significant difference within the groups (Table 3.2 b)

3.4.3: Correlation Coefficient of Serum Sodium indicated that sodium is not correlated with any parameter in male patients groups (Table 3.3a) it is however, correlated with MPS (level of

infestation) with value 0.28* and negatively correlated with urea with value -0.26* in the female patients groups.

3.4.4: Regression analysis does not revealed any relationship between serum sodium and level of malaria infestation MPS.

3.5 ANALYSIS OF SERUM POTASSIUM IN MALARIA PATIENTS AND HEALTHY INDIVIDUALS.

3.5.1: Descriptive analysis of serum potassium shows that the mean values for males and females healthy individuals are 4.33 $\pm$ 0.79 and 4.21$\pm$ 0.16. 11.1% of the male children patients have their serum potassium above normal value, same percentage have serum potassium lower than the normal value (Table 3.1a). 5.9% of female children patients have serum potassium above normal and same percentage have serum potassium below normal. The mean value is 4.05$\pm$ 0.15.Female adult patients have the mean value of their serum potassium to be 4.29%

3.5.3 Correlation matrix shows that potassium is correlated negatively with creatinine (-0.19*) and positively correlated with sodium (0.21*) in male malaria patients

3.6: ANALYSIS OF SERUM BICARBONATE (HCO3-) MALARIA PATIENTS AND HEALTHY INDIVIDUALS.

3.6.1: Descriptive analysis of serum bicarbonate in children patient show that 16.7% of the male children have elevated serum bicarbonate above normal (Table 3.1b). Male adult patients and female adult non- pregnant women have 4.8% and 10.2% of serum bicarbonate above normal (Table 3.1b).

3.6.2: One-Way analysis of variance for serum bicarbonate indicated that the mean values of the healthy individuals, children and adults for females bear the same superscript and are

therefore insignificant. However, male patient groups with mean values 26.51 ± 0.39^{a}, 28.83 ± 0.94^{b} and 25.61 ± 0.40^{b} show significant difference with the healthy individuals because of the difference of superscript the values bear (Table 3.2a).

3.6.3: Correlation Coefficient of Serum bicarbonate indicated that bicarbonate is correlated with sodium with value 0.20* in male patients groups (Table 3.3a).

3.7: ANALYSIS OF SERUM CHLORIDE IN MALARIA PATIENTS AND HEALTHY INDIVIDUALS.

3.7.1: Descriptive analysis of Female adult patients of serum chloride: The mean values of the healthy individuals are 106.35± 0.92 and 107.33± 1.33 for males and females respectively. 16.7% of the male children and 14.5% of the male adults have their serum chloride level above normal value, with mean value of 99.39± 6.06 and 10.2.56± 1.88 respectively (Table 3.1b). 17.65% of the female children, 16.33% and 33.3% of non pregnant and pregnant females patients have there respectively chloride value above normal value. Their mean values are 103.76± 2.20, 104.99± 0.93 and 104.15± 3.46 respectively.

3.7.2: One-Way analyses of variance show no significant variation in the serum Chloride mean values, of the patients and healthy individuals tested. (Table 3.2a and 3.2b)

3.7.3: Correlation coefficient: In male patients group's chloride is correlated with Sodium with value 0.21* and Bicarbonate with value 0.23* (Table 3.3a) .No correlation in the female patients group in relation to serum chloride parameter. (Table 3.3b)

TABLE 3.1a: Descriptive analysis of the parameters (proteinuria, urea and creatinine) tested on the male and female malaria patients and healthy individuals. The parameters tested and their normal (reference) values, the number and percentages of the patients in relation to the parameters that are higher, lower or within the normal values are indicated. The mean and standard deviation is also included. The result is generally based on age groups (i.e. children, adult (pregnant and non-pregnant women).

TABLE 3.1a

Descriptive Analysis of the result of male and female malarial patients (Proteinuria, Urea and Creatinine)

Parameter & Normal Values (Ref. Values)	Control		PATIENTS											DF	
	Male	Female		Children				Adult						Male	Female
				Male		Female		Male		Female					
	N = 43	N = 15		N = 18		N = 17		N = 85		Non – Pregnant N = 49		Pregnant N = 33			
	Means	± SD		No. Of patients	Percentage %	No. Of patients	Percentage %	No. Of patients	Percentage %	No. Of patients	Percentage %	No. Of patients	Percentage %		
Protein (mg/dl) 0-15mg/dl	12.00 ± 0.72	14.68 ± 1.60	> 15mg/dl	13	72.2	14	82.4	58	68.2	30	61.2	21	63.5	145	113
			≤ 15mg/dl (normal	5	27.8	3	17.6	27	31.8	19	38.8	12	36.4		
			Means ±SD	24.79 ± 4.06		33.87 ± 6.48		23.67 ±1.64		23.2 ± 2.19		24.62± 2.13			
Urea mmol/L 3.0 – 6.6	4.96 ± 0.22	4.91 ± 0.05	>6.6 mmol/L	5	27.8	6	35.3	16	18.8	4	8.2	3	9.1		
			< 3.0	8	44.4	2	11.8	12	14.1	17	34.7	10	30.3		
			Normal	5	27,8	9	52.9	57	67.1	28	57.1	20	60.6		
			Means ±SD	4.39 ± 0.56		5.54 ± 0.79		5.26 ± 0.35		3.99 ± 0.26		3.57± 0.27			
Creatinine (µmol/L) 72.0 - 126	108.49 ± 2.80	109.98 ± 6.31	>126 µmol/L	4	22.2	6	35.3	22	25.1	5	10.2	5	15.2		
			< 72	1	5.6	0	0	4	4.7	3	6.1	3	9.1		
			Normal	13	72.2	11	64.7	59	70.2	41	83.7	25	75.7		
			Means ±SD	103.44 ±7.21		119.59± 11.78		115.76 ± 4.01		99.03 ± 4.20		102.37 ± 4.45			

The table shows the ranges, number of patient and percentage of the values (parameter) that are higher, lower or within the normal value. Means and Standard deviation included:

> Greater than normal values

< Less than normal values

≤ Less or equal to the normal

± SD – standard deviation

TABLE 3.1b:Descriptive analysis of the parameters (Electrolytes: Na^+, K^+, HCO_3^- and Cl^-) tested on the male and female malaria patients and healthy individuals. The parameters tested and their normal (reference) values, the number and percentages of the patients in relation to the parameters that are higher, lower or within the normal values are indicated. The mean and standard deviation is also included. The result is generally based on age groups (i.e. children, adult (pregnant and non-pregnant woman)

TABLE 3.1b

Descriptive analysis of the result of male and female malarial patients (Electrolyte Na^+, K^+ HCO_3^- Cl^-)

Parameter & Normal Values (Ref. Values)	Control		PATIENTS											DF	
	Male	Female		Children				Adult						Male	Female
				Male		Female		Male		Female					
	N = 43	N = 15		N = 18		N = 17		N = 85		Non – Pregnant N = 49		Pregnant N = 33			
	Means	± SD		No. Of patients	Percentage %	No. Of patients	Percentage %	No. Of patients	Percentage %	No. Of patients	Percentage %	No. Of patients	Percentage %		
Sodium (Na^+) mmol/L 135 – 150	129.5 ± 1.01	126.73 ± 1.89	> 150	1	5.6	8	47.1	0	0	1.0	2.0	0	0	145	113
			< 135	11	61.1	0	0	52	61.2	24	49.0	16	48.5		
			Normal	6	33.3	9	52.9	33	38.9	24	49.0	17	51.5		
			Means ±SD	131.61 ± 2.00		133.65 ± 1.34		130.72 ±0.84		133.08 ± 1.06		133.64± 1.04			
Potassium (K^+) mmol/L 3.5 – 5.2	4.33 ± 0.79	4.21 ± 0.16	> 5.2	2	11.1	1	5.9	2.	2.4	4	8.2	1	3.03		
			< 3.5	2	11.1	1	5.9	9	10.6	3	6.1	4	12.12		
			Normal	14	77.8	15	88.2	74	87.0	42	85.7	28	84.85		
			Means ±SD	4.22 ± 0.18		4.05 ± 0.15		4.01 ± 0.07		4.29 ± 0.10		4.15 ± 0.12			
Bicarbonate (HCO_3^-) mmol/L 22 - 31	26.51 ± 0.39	26.47 ± 0.80	> 31	3	16.7	0	0	4	4.8	5	10.2	0	0		
			< 22	0	0	0	0	2	2.4	0	0	1	3.03		
			Normal	15	83.3	17	100	79	92.8	44	89.8	32	96.97		
			Means ±SD	28.83 ± 0.94		27.12 ± 0.64		25.61 ± 0.40		27.02 ± 0.42		26.09 ± 0.42			
Chloride (Cl^-) mmol/L 97 - 108	106.35 ± 0.92	1.07.33 ± 1.18	> 108	3	16.7	3	17.65	12	14.5	8	16.33	11	33.3		
			< 97	1	5.6	3	17.65	5	6.02	4	8.16	2	6.06		
			Normal	14	77.8	11	64.7	68	79.98	37	75.51	20	60.61		
			Means ±SD	99.39 ± 6.06		103.76± 2.20		102.56 ± 1.88		104.49 ± 0.93		104.15 ± 0.42			

The table shows the ranges, number of patient and percentage of the values (parameter) that are higher, lower or within the normal value. Means and Standard deviation included:

> Greater than normal values

< Less than normal values

≤ Less or equal to the normal

± SD – standard deviation

The descriptive analysis of both sexes of the parameters analyzed in Table 3.1a and Table 3.1b shows the variation between the male and female sexes. The mean values for the proteinuria of male and female children differ significantly with that of the healthy individuals. Significant variation is also indicated in thee female gender with the female children having higher variation .it is also noted that gender variation gives a possible contribution to the variation.

Table 3.2a : One- way analysis of variance of the parameters tested for male malaria patients(children and adults) and healthy individuals. Standard error of means (± S.E.M) is indicated. Data in the same row carrying different superscripts (**a, b,** or **ab**) differ significantly from each other.

Table 3.2b : One- way analysis of variance of the parameters tested for female malaria patients(children and adults (non-pregnant and pregnant women)) and healthy individuals. Standard error of means (± S.E.M) is indicated. Data in the same row carrying different superscripts (**a, b,** or **ab**) differ significantly from each other.

TABLE 3.2 a

ONE-WAY ANALYSIS OF VARIANCE FOR MALE MALARIA PATIENTS

PARAMETERS	CONTROL	CHILDREN	ADULT	± S.EM
MalariaParasite (Mps)	0.00 ± 0.00^{a}	2.00 ± 0.12^{b}	2.00 ± 0.08^{b}	± 0. 09
Protein	12.00 ± 0.72^{a}	24.79 ± 4.06^{b}	23.67 ± 1.64^{b}	± 2. 19
Urea	$4.96 \pm 0.22a$	4.39 ± 0.56^{a}	5.26 ± 0.35^{a}	± 0. 45
Creatinine	108.49 ± 2.80^{a}	$10.3.44 \pm 7.2^{a}$	115.76 ± 4.01^{a}	± 5. 27
Sodium (Na^{+})	129.51 ± 1.01^{a}	131.61 ± 2.00^{a}	130.72 ± 0.84^{a}	± 1. 25
Potassium (K^{+})	4.33 ± 0.79^{b}	4.22 ± 0.18^{ab}	4.01 ± 0.07^{a}	± 0.11
Bicarbonate (Hco_3^{-})	26.51 ± 0.39^{a}	28.83 ± 0.94^{b}	25.61 ± 0.40^{a}	± 0.57
Chloride (Cl^{-})	106.35 ± 0.92^{a}	99.39 ± 6.06^{a}	102.56 ± 0.40^{a}	± 2.70

Data in the same row carrying different super scripts (i.e. a, or b or ab) differ significantly from each other therefore($P<0.05$)

TABLE 3.2 b

ONE-WAY ANALYSIS OF VARIANCE FOR FEMALE MALARIA PATIENTS

Parameters	Control	Children	Non–Pregnant (Adult)	Non–Pregnant (Adult)	± S.EM
Malaria Parasite (Mps)	0.00 ± 0.00^{a}	2.00 ± 0.19^{b}	2.00 ± 0.11^{b}	2.00 ± 0.14^{b}	± 0.15
Protein	14.68 ± 1.60^{a}	33.87 ± 6.48^{b}	28.21 ± 2.19^{ab}	24.62 ± 2.13^{ab}	± 3.26
Urea	4.91 ± 0.55^{ab}	5.54 ± 0.79^{b}	3.99 ± 0.26^{a}	3.57 ± 0.27^{a}	± 0.42
Creatinine	109.98 ± 6.31^{a}	119.59 ± 11.73^{a}	$99.03 \pm 4..20^{a}$	102.37 ± 4.45^{a}	± 6.42
Sodium (Na+)	126.73 ± 1.89^{a}	133.65 ± 1.34^{b}	133.08 ± 1.06^{b}	133.64 ± 1.04^{b}	± 1.35
Potassium (K+)	4.21 ± 0.16^{a}	4.05 ± 0.15^{a}	4.29 ± 0.10^{a}	4.15 ± 0.12^{a}	± 0.14
Bicarbonate (HCO3)	26.47 ± 0.08^{a}	27.12 ± 0.64^{a}	27.02 ± 0.42^{a}	26.09 ± 0.42^{a}	± 0.57
Chloride (Cl)	107.33 ± 1.18^{a}	103.76 ± 2.20^{a}	104.49 ± 0.93^{a}	104.15 ± 3.46^{a}	± 2.49

Data in the same row carrying different super scripts (i.e a, or b or ab) differ significantly from each other therefore($P<0.05$)

The result of one-way analysis of variance for male and female malaria patients in Table 3.2a and 3.2b show that, the data in the same row carrying different superscripts differ significantly ($p<0.05$) from each other. Significant different ($p<0.05$) is noted between ***mps*** for the healthy individuals and that of the patients, which indicate the presence of malarial parasites in patients and absent in the healthy individuals.

In table 3.2a the mean value for the protein concentration in urine of male healthy individuals children and adult groups are 12.00 ± 0.72^{a}, 24.79 ± 4.06^{b} and 23.67 ± 1.64^{b} respectively. healthy individuals group value differ significantly from that of the children and adult group, since the mean values bear different superscripts ***a*** and ***b***. Children and adult group on the other hand are insignificant, since the values are carrying same super script ***a.***

Serum Potassium (K+) mean values show significant variation ($p<0.05$) between the male patients group and the healthy individuals group. Significant variation is also indicated in serum bicarbonate between male children patients and the healthy individuals group.

Table 3.2b show that the mean values of the 3 groups of female patients vary significantly ($p<0.05$) with the healthy individuals ,While the children group differ significantly from all the groups, adult groups (pregnant and non pregnant) are insignificant ($p>0.005$). Serum urea values also indicated the same variation in the groups. Significant variations are also indicated in serum sodium between female patients and the healthy individuals, but insignificant, ($p>0.05$) within the male patient groups.

From the tables, there is indication of gender variation in relation to the parameters tested. Proteinuria is indicated in the 2 sexes, urea and sodium (Na+) in female groups. Potassium (K+) is indicated with male groups only and other parameters are insignificant, male and females alike.

Table 3.3a and 3.3b: The correlation coefficient (Matrix) of the parameters tested for male and female malaria patients (children and adults (non-pregnant and pregnant women)). The values carrying star (*) indication are correlated. The correlation could be positive or negative.

TABLE 3.3 a

CORRELATION MATRIX FOR MALE MALARIA PATIENTS

Y = Correlation coefficient

N = 146

	MPs	Protein	Urea	Creatinine	Na^+	K^+	HCO_3^-	Cl^-
MPs	-							
Protein	0.32*	-						
Urea	-0.001	-0.06	-					
Creatinine	0.01	0.03	0.85*	-				
Na^+	0.06	0.09	0.03	0.03	-			
K^+	0.16	0.10	0.14	-0.19*	0.21*	-		
HCO_3^-	-0.02	0.04	0.06	-0.12	0.20*	0.05	-	
Cl^-	-0.11	0.04	0.07	0.6	0.21*	0.11	0.23*	-

* P< 0.05: Those parameters that their values are indicated by * are correlated. (-) Sign indicates negative correlation

Note: 0.31* show that MPs and Protein are positively correlated

TABLE 3.3 b

CORRELATION MATRIX FOR FEMALE MALARIA PATIENTS

Y = Correlation coefficient

N = 114

	MPs	Protein	Urea	Creatinine	Na^+	K^+	HCO_3^-	Cl^-
MPs	-							
Protein	0.23*	-						
Urea	-0.21*	-0.01	-					
Creatinine	-0.14	0.03	0.78*	-				
Na^+	0.28*	0.06	-0.26*	0.16	-			
K^+	-0.03	0.04	-0.05	-0.14	0.11	-		
HCO_3^-	0.11	0.10	0.04	-0.02	0.16	0.27	-	
Cl^-	-0.07	-0.01	0.08	0.13	0.06	0.04	0.09	-

* P< 0.05 i.e correlated : Those parameters that their values are indicated by * are correlated. (-) sign indicates negative correlation

Note: - 0.21* show that MPs and Urea are negatively correlated

These tables show how parameters tested are correlated in male and female malaria patients. The values carrying star (*) indication shows correlation ($p<0.05$) between the parameters. The tables show that protein/Mps (0.32* and 0.23*) and urea/creatinine (0.85* and 0.78*) are correlated in both sexes, male and females respectively.

Potassium /sodium (0.21*), bicarbonate /sodium (0.23*), sodium/chloride and chloride/ bicarbonate are positively correlated in male groups (Table 3.3a) .In female groups sodium is positively correlated with Mps (0.28*) and negatively correlated with urea (- 0.26*) (table 3.3b). All these are possible indication of gender variation. By positive correlation it means when one parameter increases the other increase correspondingly, while negative correlation means if one parameter increase the other decrease correspondingly.

Table 3.4: Is the regression analysis (model fitting) for the parameters tested for male and female malaria patients; in relation to the level of infestation.

TABLE 3.4

MODEL FITTING (MALARIA PARASITES MPs) and other parameters

PARAMETERS	MALE	FEMALE
Protein	0.02*	0.01*
Urea	0.01	-0.08
Creatinine	0.001	0.001
Na^+	0.01	0.03*
K^+	0.20	-0.13
HCO_3-	0.002	0.03
Cl	0.01	-0.004

Table 3.4 shows the relationship between parasitaemia (MPs) and other parameters in male and female patients.

* Indication on the value show ($P<0.05$) i.e. the parameter's dependency on Mps is significant. Therefore relationship exists between Mps and such parameter.

Formula

$$Y = a + b_1 x_1 + b_2 x_2 + b_3 x_3 + b_4 x_4 + b_5 x_5 + b_6 x_6 + b_7 x_7 + E$$

Y = MPs

X_1 = Protein

X_2 = Urea

X_3 = Creatinine

X_4 = Sodium

X_5 = Potassium

X_6 = Bicarbonate

X_7 = Chloride

a = Constant

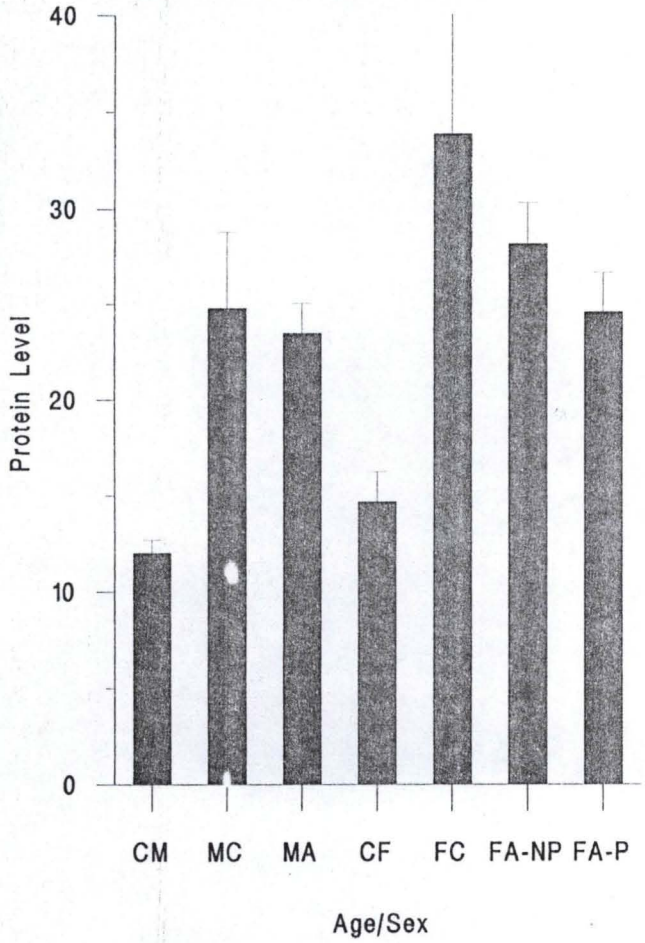
b = Coefficients

E = dub wart constant

The regression analysis in table 3.4 shows how malaria parasite infestation and other parameters are related in male and female patients, that is, those parameters whose variation are dependent on the level of infestation.

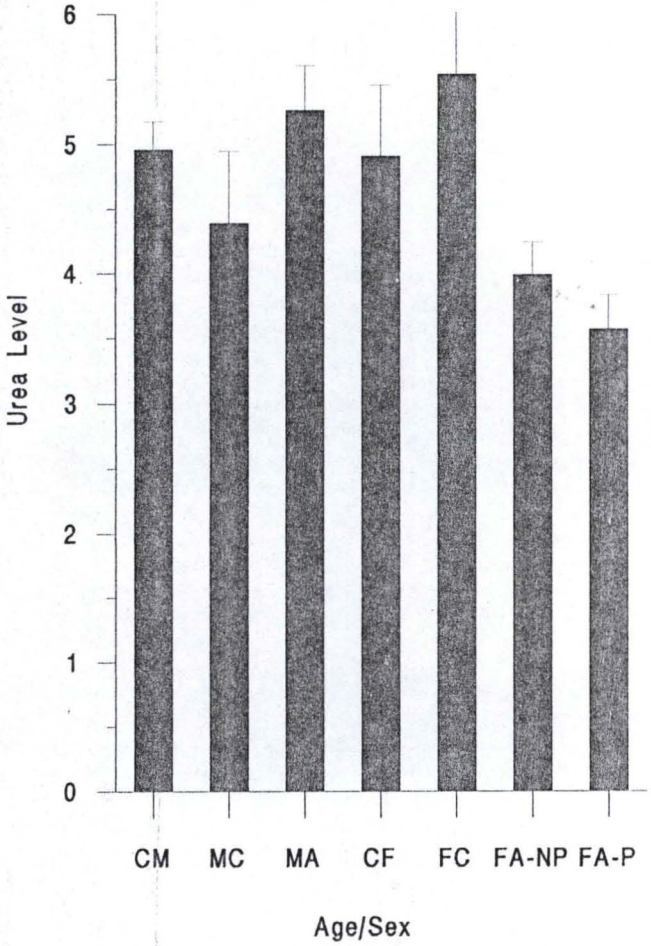
It is indicated from the table that proteinuria values for male (0.02*) and female (0.01*) are as a result (dependent) of malaria parasitamea. ($p<0.05$) sodium (Na^+) value (0.03*) in female groups only.

Fig.3.1:The level of serum protein in malaria patients of different age groups and sex



CM-male control
MC-male child
MA-male Adult
CF-Female control
FC-Female child
FA-NP-Female Adult non-preg
FA-P- Female pregnant

Fig.3.2:The level of serum urea in malaria patients of different age groups and sex



CM-male control

MC-male child

MA-male Adult

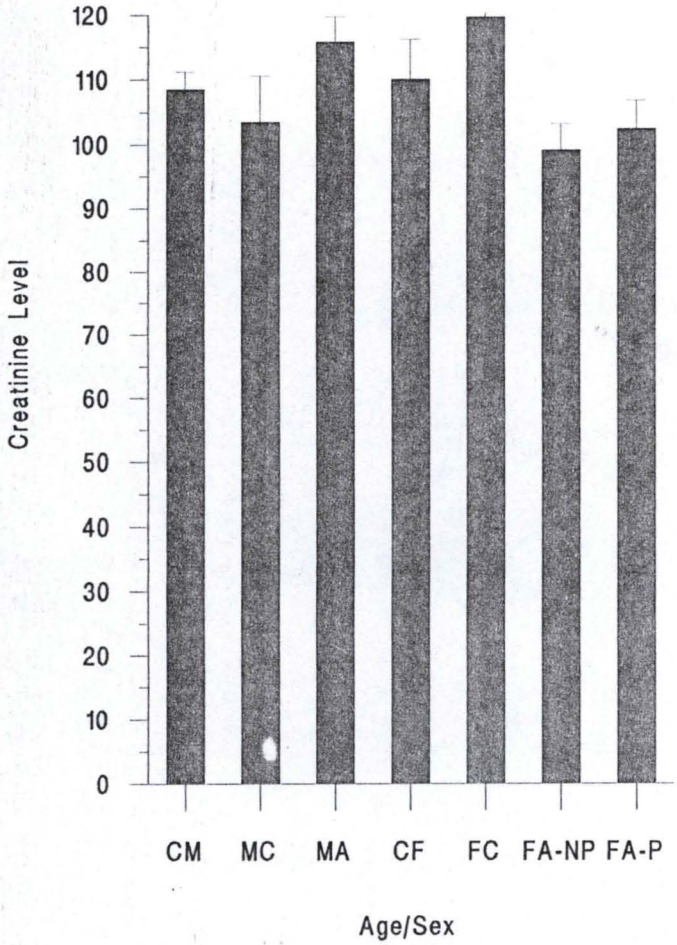
CF-Female control

FC-Female child

FA-NP-Female Adult non-preg

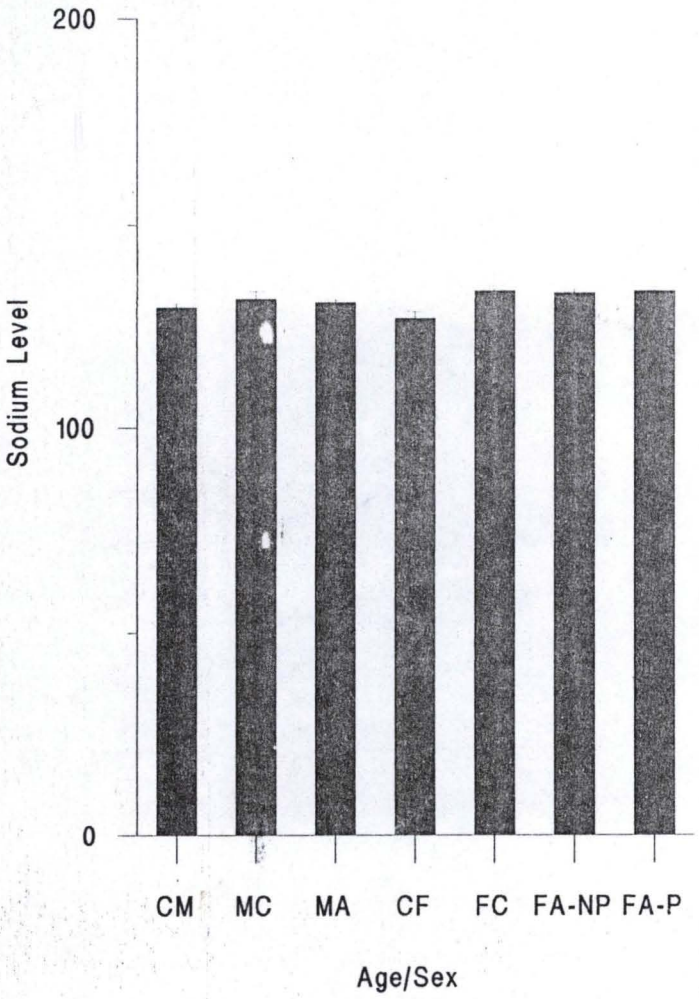
FA-P- Female pregnant

Fig.3.3:The level of serum creatinine in malaria patients of different age groups and sex



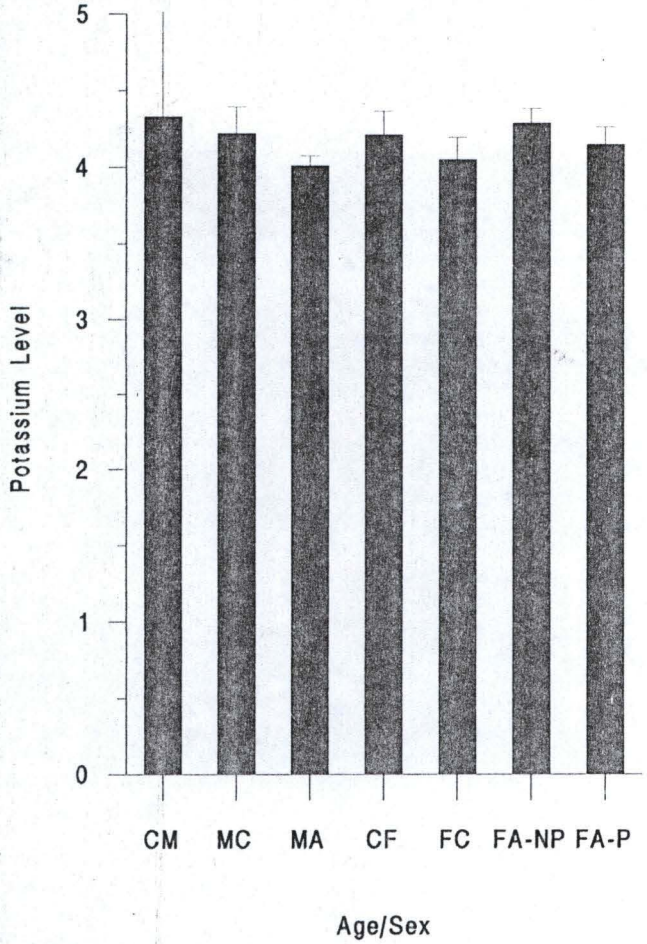
CM-male control
MC-male child
MA-male Adult
CF-Female control
FC-Female child
FA-NP-Female Adult non-preg
FA-P- Female pregnant

Fig.3.4:The level of serum sodium in malaria patients of different age groups and sex



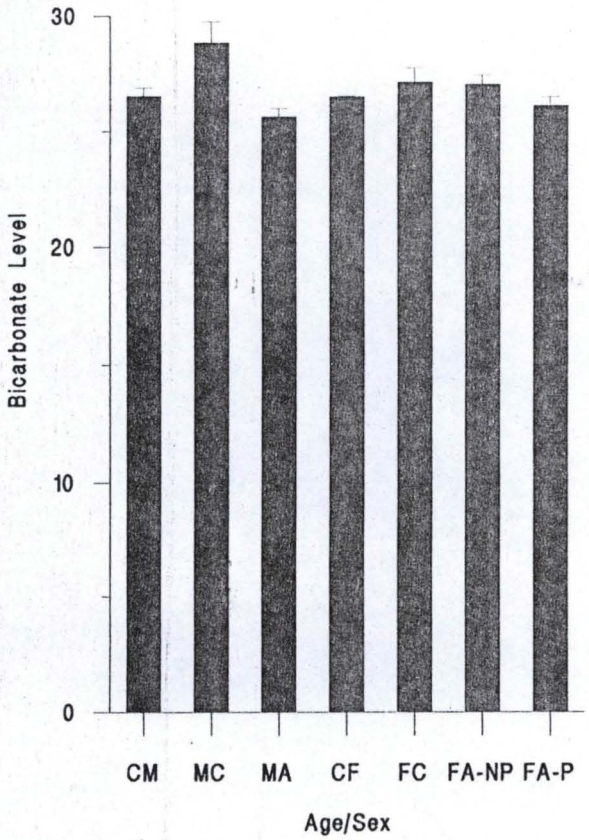
CM-male control
MC-male child
MA-male Adult
CF-Female control
FC-Female child
FA-NP-Female Adult non-preg
FA-P- Female pregnant

Fig.3.5:The level of serum potassium in malaria patients of different age groups and sex



CM-male control
MC-male child
MA-male Adult
CF-Female control
FC-Female child
FA-NP-Female Adult non-preg
FA-P- Female pregnant

Fig.3.6:The level of serum bicarbonate in malaria patients of different age groups and sex



CM-male control

MC-male child

MA-male Adult

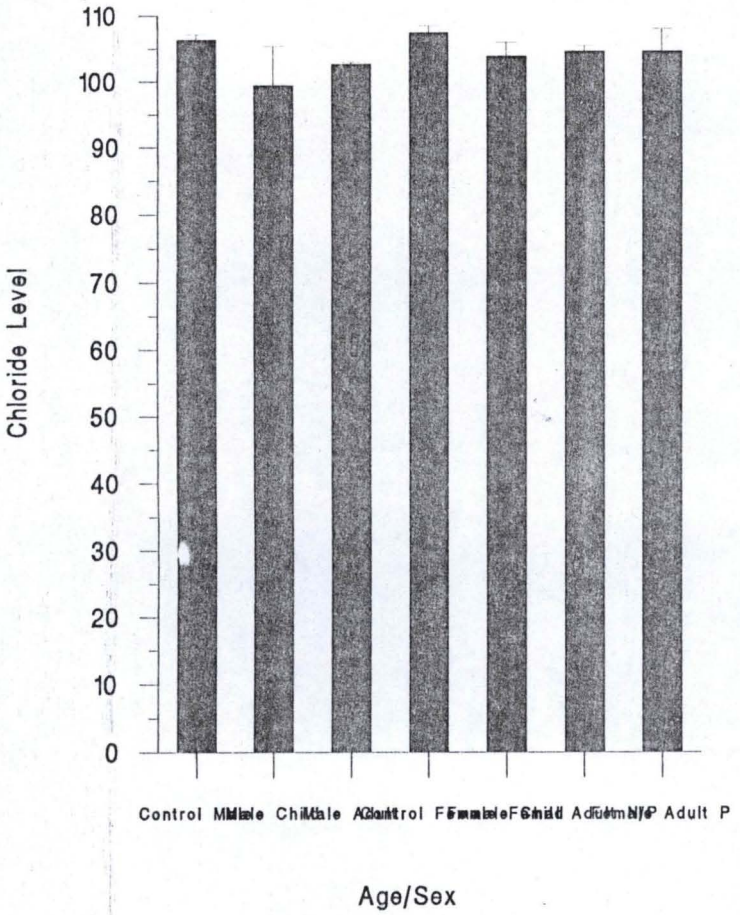
CF-Female control

FC-Female child

FA-NP-Female Adult non-preg

FA-P- Female pregnant

Fig.3.7: The level of serum chloride in malaria patients of different age group and sex



CM-male control
MC-male child
MA-male Adult
CF-Female control
FC-Female child
FA-NP-Female Adult non-preg
FA-P- Female pregnant

FIG 3.1 –3.7: Represent the graphic representation of parameters analyzed, the concentration is plotted against AGE/SEX

CHAPTER FOUR

DISCUSSION AND CONCLUSION

DISCUSSION

It has been reported that nephritic syndrome and acute renal failure in severe malaria is common, occurring as complication in less than 1% of cases in *P. falciparum* and *P. malariae* (Abdurahman et al, 1983 a; Mishra, 2002). The mortality rate in cases of *P. falciparum* as it relates to renal failure is about 45% (Mishra, 2002). The present study has shown some abnormalities in kidney function in patients with malaria. Higher incidence of proteinuria, a characteristic feature of renal dysfunction (Rui – mei, 1998b) was noted in 61.2–82.4% of the total cases studied. This is relatively higher when compared to the earlier record of 52% (Ogbadoyi and Tembeng, 1999). A related report by BoonPucknaviq; (1979) showed about 20 – 50% proteinuria in malaria cases. All these are indication of possible increases in the prevalence of renal failure in malaria cases in endemic areas. This research showed significant increase in the level of proteinuria for the malaria patients groups when compared with that of non-malaria individuals ($P<0.05$) .The total result indicated that 67.33% of the total patients had values over 15 mg/dl while 32.67% had values less than 15 mg/dl. It was

observed that gender variation was statistically insignificant in relation to the parasite infestation. However, strong correlation was noted between the level of proteinuria and the level of parasitaemia. The level of proteinuria was higher in children compared to the adults and highest in female children. Hence increases the chances of being prone to possibility of kidney dysfunction. Proteinuria level in adult female on the other hand was also higher as compared to the adult males, which is a clear demonstration of gender differences, as speculated in previous studies (Ogbadoyi and Tembeng, 1999). Such variations may not be unconnected with the anatomical composition that may predispose females to urinary and genital tract infection (UTI and GTI), which could likely contribute to such a significant increase in the level of proteinuria (Abdurrahman et al, 1983 b). Further research is however, required to rule out possible UTI and GTI infections.

It is also possible that vaginal discharges could contribute to high proteinuria. One could argue that the classes of females that are prone to such infection (UTI, GTI and possible vaginal discharges) are the adult females, especially pregnant women. Since Hormonal influences during pregnancy reduce urinary tract contraction with resultant dilation and reduction in peristalsis of the tract. This increases the chances of being more susceptible to infection. In addition covert or asymptomatic

bacteriuria could contribute to high proteinuria (Edward and Bouchier, 1991), surveys indicated that approximately 1% of children under the age of 1, about 1% of school girls, 0.03% of school boys and men, about 3% of non–pregnant adult women and 5% of pregnant women have covert bacteriuria (Edward and Bouchier, 1991). No evidence so far, shows that covert or asymptomatic bacteriuria could lead to chronic interstitial nephritis, however, possible high level of proteinuria could result, especially in the adult female groups. Despite all these, the result indicated less significant variation between the male children and the pregnant women and insignificant variation when compared with non–pregnant women (Mean Proteinuria $P>0.05$). It is worth mentioning, that in apparently healthy persons, usually children or adolescent, small amounts of protein are excreted without demonstrable renal disease, a condition known as postural or orthostatic proteinuria (Edward and Bouchier, 1991). But even with this, the level should not be significantly high as noted. Therefore, the higher level of proteinuria recorded is an indication of probable higher pre-disposition of female children to renal failure in condition of malaria. To this end, it is strongly recommended that studies be designed to further investigate gender variation as it relates to the incidence of proteinuria and the possible predisposition of female children to renal dysfunction in severe malaria complications.

Regression analysis further revealed the direct link (dependency) of proteinuria with parasite infestation, clearly indicating malaria contribution in renal dysfunction.

Serum urea level variation was not significant in male groups in this research finding (P>0.05). Urea was said to be a less reliable guide to the overall renal functions, since it can be more readily affected by dietary protein, tissue breakdown and hydration "(Edward and Bouhier, 1991). Never the less, it is known that neither plasma creatinine nor blood urea rises above the maximum, until renal function is reduced by at least 50% (Edward and Bouchier, 1991). So from the result (Table 3.2a) despite the insignificant variation (P>0.05) in the analysis of male malaria patients, descriptive analysis (Table 3a) shows that about 18-28% have urea level above 7.0mmol/L, which is an important indication of renal dysfunction. Significant variation was noted in urea level of the female classes. About 35.5 % of female children (Table 3b) have urea level above 7.0mmol/L (Mean 5.54 $\pm$ 0.79) compared to the healthy individual (mean 4.91$\pm$ 0.55). This gives a strong indication of there being more susceptible to acute renal failure due to malaria.

A striking point to note, is that the Significant variation noted in urea level, indicated negative correlation between proteinuria and serum

urea (Table 3. 3b) i.e. when protein concentration in urine is high, the serum urea level is significantly low, an irony of what is obtained in clinical condition, in which both should be proportional. This is clear from {Table 3.2b} where the serum urea levels of non–pregnant and pregnant women (mean 3.99 + 0.26 and 3.37 + 0.27 respectively) were lower than that of the healthy individual (means 4.91± 0.55). This observation could be explained in two ways.

i. Low protein intake or malnutrition: Large number of our people drinks pap, locally prepared from corn, as their main meal when they are ill. This coupled with metabolic disorders such as hydration and liver damage in severe complicated malaria (Cheesbrough, 1991a; Edward and Bouchier, 1991, and Thomson and Connor, 2001) could result in low serum urea level in malaria patients.

ii. Increased urinary excretion of urea: uremia (a marked increase in blood urea level) is one of the pre–renal clinical manifestations of acute renal failure (ARF) (Mishra, 2002). But as it progresses the kidney losses its counter current mechanism and this is follow by a sudden increase in the urinary excretion of the urea with a negative resultant effect on the serum level of urea,

making it lowers than the normal level (Edward and Bouchier, 1991).

Previous studies have shown that elevated serum creatinine level was detected in malaria patient, up to about 265µmol/L in adults and above 62µmol/L in children (Stone et al, 1972 and Weber et al, 1999). Similar observation was noted in this work.

About 35 % of female children (means 119.59±11.78) and 25 % of the adult male (means 115.76 ± 4.01) had elevated serum creatinine level above 130µmol/L and up to a maximum level of about 285µmol/L in adult. The level of serum creatinine is above normal, which is an indication of kidney dysfunction. It is however, noted that despite the higher level of creatinine as reported above, the creatinine level variation in this finding is statistically insignificant between the patient groups and the healthy individuals ($P>0.05$.) In addition, no significant correlation was noted between the serum creatinine, parasitaemia and urinary protein level. However, strong and positive correlation exists between the serum creatinine and the serum urea level (Table 3.3a). The contributing factors that might be responsible for such statistical insignificant variation include:

1. Diurnal variation (variation in creatinine level associated to the day's activities of the individual). Higher level of muscle activities or muscle wasting in the day bring about elevation of the serum creatinine which is especially true in healthy individual, whose activities are not restricted as in the case of the patient. Hence false positive result could be an outcome.

2. High level of ketone bodies in the serum of individual that starved for very long period could also interfere with the creatinine level giving false positive result (Cheesbrough, 1991b). However, the strong positive correlation exhibited between urea and creatinine.Level in the result (table 3.3b) ($P>0.05$) is suggestive of a strong indication of possible renal involvement in acute malarial cases.

There is indication of hyponatremia (low serum level of sodium) in this work when compared to the standard normal reference value (135–150 mmol/L). 61% of the total cases have low sodium value between 120–134mmol/L; earlier studies had reported similar observation of hyponatremia in about 67% cases in patients with severe malarial infection (BoonPuknaviq, 1979). However, statistically it is observed that the variation is insignificant in serum sodium level of male healthy

individual group and the patients groups. No significant correlation between sodium and malarial parasitaemia in male groups. Female groups on the other hand, showed significant variation ($P<0.05$) between healthy individuals and patient groups (table 3.2b).

Possible aetiology to hyponatremia in malaria infestation as explained by (Boon Puknaviq et al, 1979) were evidence of sodium depletion, increased anti diuretic hormone (ADH) activities due to hypervolemia, high urine sodium concentration, hyper-osmotic urine and normal renal function. Possible mechanism responsible for inconsistency in the secretion of ADH was not feasible, but related to hyperpyrexia. In some cases hyponatremia was unexplained but related to possible "resetting" of the osmotic receptors. The variation in the normal standard value for Na^+ (i.e. 95 – 150mmol/L) as used in the General hospital where the test was conducted could be associated with a known fact that, the normal standard value for electrolyte, generally could vary among institutions and depend on the testing methods used (Sheryl and Stark 1997; Bray et al; 1999). Moreover, it is currently reported that the effect of renin–angiotensin aldosterone system in the regulation of glomerular filtration rate and tubular re–absorption of Na^+ was found to be low in black race (Africans) compared to the other classes of people (e.g. cancans). These are possible explanations for the variation in the

normal range; however, more work may be required to further elucidate the significant variation and positive correlation evidenced in female malaria patients.

This research work showed that considerable significant variation was recorded in the male groups between the healthy individuals and patient groups, for the serum potassium concentration. Despite the fact that majority of the values obtained were within the normal ranges. However, report has shown that hyperkalamia is recorded in malarial related renal failure and other parasitic infections, up to 6.5mmol/L and above (Williams and Baltimore, 1972; Abdurahman et al, 1983b; Parmar, 2002). In a related finding, significant correlation was observed within the electrolyte for the male groups, but was insignificantly correlated within the female groups. One could not say really whether it was the gender difference that brought about such difference. Literature materials on the effect of malaria on chloride and bicarbonate seem to be scarce as at the time this report was compiled.

However, our findings did not show any significant variation and therefore no probable effect was indicated on the serum chloride and bicarbonate.

CONCLUSION

Malarial infection is a threat to humanity and the incidence as well as organ involvement, such as the kidney is increasing at alarming rate. Report of anti malarial resistance in *P. falciparum* and other species is becoming higher and could contribute immensely to the possible complication of renal failure. However it could concluded from this work that:

1. Possible renal involvement in acute malaria is noted.
2. Gender variation as it relates to renal dysfunction in acute malaria is also noted

It is therefore pertinent to mention here, that early recognition and proper management is required. Moreover, any complicated malaria case should be examined for possible renal involvement. Multiple complications need urgent management in a tertiary care hospital with multi–disciplinary approach. It is recommended that wider studies, which will look at several aspects of this subject area at the same time, are required. Such research finding could probably give an in-depth understanding of the renal failure and its patho- mechanism in malaria cases as it relates to sexes and age groups.

REFERENCES

Abuja 2000 Abuja dedication on Roll back malaria in African, by the African Heads of States and Government; April 25, 2000 Nigeria

Abdurrahman M B.; Green Wood BM; Drape CC; Edington GM;

Narayana PT; Babaoye FA; and Mclaren MC, 1983a: The role of malarial in childhood nephritic syndrome in Northern Nigeria. East African Medical Journal. 60(7), 467 – 471

Abdurrahman MB; Onuora CU, Babaoya FA; and Narayana PT; 1983b Renal failure in children in Northern Nigeria. East African Medical Journal 60(7), 472 – 477

Adams HJ 2001.Molecular and cell biology of parasitic Protozoa 1 – 2

Boonpucknaviq V and Sitprija V 1979. Renal disease in acute *plasmodium falciparum* infection in man Kidney international 6:44 – 54

Bray JJ ;Cragg AP, Macknight ADC, and Mill RG, 1999. Lecture notes on Human physiology 4thedition, University Press, Cambridge United Kingdom. Pp 2 - 8

Cheesbrough M. 1991a Medical laboratory manual for tropical countries, University press Cambridge Great Britain 1: 221 – 251

Cheesbrough M. 1991a Medical laboratory manual for tropical countries, University press Cambridge Great Britain 1: 221 – 251

Cheesbrough M. 1991b Medical laboratory manual for tropical countries, University press Cambridge Great Britain 2: 133-160

Chris C; Lines JO and Daniel C; 1997.Preventing malaria, International newsletter on child health and disease prevention; child health dialogue 1st quarter 6: 4- 5

Connor S. 2001.Deadly Malaria may have arisen with spread of Agriculture. National Geographic News Article. The independent London

Carrington A, 2001.Malaria; its Human impact challenges and control strategies in Nigeria – Fall in Focus 2 (2): 1 –3

David son and Henry, 1979; Clinical diagnosis by laboratory method 17th edition, ELBS New York Pp340 – 500

Dobson M and Snow R. 2000 A History of malaria and its control in 20th Century East Africa welcome unit for the History of medicine.

Dipalma RJ, 1971 .Drill's pharmacology in medicine 4th Ed. Mcaraw hill book company, a blakistion publication New York.Pp 480 – 483

Edwards M.J and Bouchier I.A.D 1991.Davidson principle and practice of medicine 16th ed. ELBS Churchill living stone man group Ltd. Hong long. Pp600 -745

Fletcher JR,Tom Butter Lt,Kopriva JC and Ratlift JL 1972 .Acute *plasmodium falciparum* malaria, vital capacity, blood gases and coagulation. Archieve international medicine 129:617 – 619

Genetic Medicine 2002.Tackling malaria through genomic 1 – 3

Glasgow E.F; Adeniyi A, Edington G.M, Hendrickse R.G, White R.H.R and Houba V. 1972. Ouartan malarial nephrotic syndrome. The lancet:

1143 – 1148

Gray CH; and Howorth PJN; 1982.Clinics chemical pathology; 9th ed. The English language Book society and Edward Arnold (publisher) Ltd.Pp2-10

Heinemann A.S and Philadelphia M.O, 1972 .The clinical syndrome of malaria in the United States. Archive international medicine129:607– 16

Houba V. 1979 .Immunologic aspects of renal lesions associated with malarial, kidney international 16: 4 – 8

Hutt M.S.R, 1979. Introduction, Kidney international 16:pp 1- 3

Karin L, and Peter FW, 2000.Treatment of malaria, up to date. 1 – 12

Kreier JP, 1980.Malaria pathology, vector studies and culture Academic press London 2: 47 – 80

Kurtis JAL; Goka OB; ,Akanmori BD and Vid H; 2001 .The importance of strict patient definition in studies of malaria pathogenesis: Trend in parasitology 17(7):313 – 314

Luxemburger C, 1997. Managing malaria. The international newsletter on child health and disease prevention. Child health Dialogue. 1st quarter 6: 8- 9

Malaria 2000 .Malaria incidence rate LAC and US. Pp 81 – 85

Malaria 2001 .Disease type blood and Hearth. 1-3

Marsh K. 1997 .Malaria, an over view; the international Newsletter on child health and disease prevention. Child health dialogue 1st quarter 6:3

Marsh K. And Snow RW. 1995. Epiodemiology of malaria in Africa presented at the 1st AMVTN meeting Arusha Tanzania: 1- 2

Meek S. 1997 Controlling malaria. Health action. The international news letter on implementing primary health care. Child health dialogue 1st quarter 17: 1

Mishra S'K, Mohapatra S, Mohanty S,; Patel NC, and Mohapatra DN, 2002. Acute renal failure in falciparum malaria journal Indian academy of clinical medicine 3(2) 141 – 147

Molyneux M. 1997 .Recognizing malaria the international newsletter on child health and disease prevention. Child Health Dialogue. 1st quarter 6: 6

Mukherjee AP, White S.L and Lau KS, 1971. falciparum malaria associated with jaundice: Renal failure and anemia. Transaction of the Royal society of tropical medicine and hygiene 65(6) 804 – 14

Ogbadoyi EO and Tembeng FC, 1999. Proteinuria in malaria patients in Minna Nigeria Journal Protozoal research 9:49 – 52

Parmar MS, 2002. Acute glomerulo nephritis medicine journal.3(8)41 –4

Polozok E.S. And Loban KM;1989.Malaria.Mir Publisher 2 pervy Rizhsky Perculok Moscow. Pp11-70

Prakash J, Gupta A, Kumar O, Rout SB, Malhotra V and Srivastava PK 1996. Acute renal failure in falciparum malaria increasing prevalence in some areas of India–a need for awareness. Nephrol Dial Transplant 11(12) 2414 – 6

RBM 1999.Roll Back malaria increasing the momentum. World health organization Genera, Switzer land.1 – 3

Reed K. 1997.Malaria in pregnancy the international news letter on child health and disease prevention child health dialogue. 1s^{t} quarter 6:9

Rees PH, Barr RD,Cordy PE and Voller A 1972 .Possible role of malaria in aetiology of the nephritic syndrome in Nairobi British journal 2;130 – 31

Roberts D and Campbell H. 1997; Malaria a continuing threats the international news letter on child health and disease prevention. Child health dialogue 1st quarter 6: 1

Robinson TA; 2000. Malaria in endemic human populations, clinical disease, immunity and protection, micro biology and immunology no – line1 – 7

Rui – mei L, Kara AU,And Sinniah R. 1998a .In situ analysis of adhesion molecule expression in kidney infected with murine malaria; Journal pathology 185(2). 219 – 25

Rui – mei L, Kara AU, And Sinniah R. 1998b.Dysregulation of cytokine expression in tubulointerstitial nephritis associated with murine malaria. Kidney international, 53(4) 8445 – 52.

Rui – mei L, Kara AU,And Sinniah R. 1998c.Up regulation of major his to compatibility complex (MHC) antigen in nephritis associated with murine infection. Journal pathology 185(2) 212 - 18

Russel PF, West LS, Manwell RD and Macdonald G. 1963. Practical malariology, 2nd Ed. Oxford University press London.Pp11 – 90

Sheryl Al and Stark LJ, 1997; Fluid and electrolytes. 3rd ed. Spring house Co-corporation, springhouse Penn sylvan USA. Pp 24 – 60

Singh N, Shukla M.M, and Sharma VP. 1999. Epidemiology of malaria in pregnancy in central India. Bulleting of the world health organization 77(7) 567 – 72

Singh SP. 1990. Manual of practical Biochemistry with latest Advance, CBS publishers and Distributors Shahdra Delhi. Pp199 – 202

Sinniah R, Rui – mei L,and Kara AU, 1999.Upregulation of cytokines in glomerulonephritis associated with murine malaria infection. International journal Exp. Pathol. 80(2) 87 – 95

Stone WJ, Hancheh JE,And Knep shield H.J. 1972.Acute renal insufficiency due to falciparum. Archive international medicine 129; 620 – 6

Thomson CM and Connor SJ, 2001,The development of malaria early warning systems for African. Trends in parasitology. 17(9) 438 – 445

Tilkian MS, Conover MB Tilkian AG. 1979. Clinical implications of laboratory. CV mosby company London.Pp 1 –15

Tiger H WD and Baltimore MD; 1972.The malaria problem, past, present and future. Archive international medicine 129. 604 – 6

Townend M; 1999 .Malaria Pp1 – 5

Trigg PI and Rietreid AEC, 1997.Treating malaria the international newsletter on child health and disease prevention, child health Dialogue. 1st quarter 6: 11.

Van Velthysen MLF 1996. Glomerulopathy, associated with parasitic infections. Parasitology today 12(3) 102 – 107

Voller A, Draper CC,Shwe TIN and Hutt MSR; 1971. Nephritic syndrome in monkey infected with quarter malaria British medical Journal 4: 208 – 1

Weber MW; Zimmer Mann U; Van Hens Brock MB; Frenkil J;Palmer A, Ehrich JHH and Green wood BM; 1999. Renal involvement in Gambian children with cerebral or mild malaria. Tropical medicine and international health 4(5) 350 – 94

White R.H.R; 1973. Quarter Malaria nephrotic syndrome. Nephron 11: 147 – 162

WHO 1986 Immunology of malaria, Report of a WHO scientific group, WHO technical report series 396: 35 – 38

WHO 1983 Terminology of malaria and malaria eradication; Report of a Drafting committee p 116

William DT and Baltimore MD 1972 The malaria problem past present and future, Archive international medicine 129:604 - 606

APPENDIX 1A

SUMMARY OF THE PREPARATION OF DIFFERENT CONCENTRATION OF BOVINE SERUM ALBUMINE (30% BSA) FOR CALIBRATION

Reagent	Blank	Test Tubes					
	(b)	1	2	3	4	5	6
De ionized Water (ml)	1.5	0.3	1.1	1.0	0.9	0.7	0.5
Standard (BSA) Img / ml	-	0.2	0.4	0.5	0.6	0.8	1.0
Biuret reagent (ml)	6.0	6.0	6.0	6.0	6.0	6.0	6.0

The test tubes were swirled to mix the contents well; and they were allowed to stand for 30mins for colour development

APPENDIX 1B

STANDARD CALIBRATION CURVE USING BOVINE SERUM ALBUMINE (30% BSA) PREPARED TO 1mg/ml i.e. 100mg/dl

Conc./mg/ml	Absorbance i	Absorbance ii	Ave. Absorbance
0.2	0.009	0.011	0.010
0.4	0.021	0.019	0.020
0.5	0.024	0.026	0.025
0.6	0.028	0.032	0.030
0.8	0.040	0.040	0.040
1.0	0.050	0.050	0..050

Linear Calibration

If straight line can be obtained through the point plotted then absorbance is directly proportional to the concentration. The Beer Lambert law applies and the followings formula can be used.

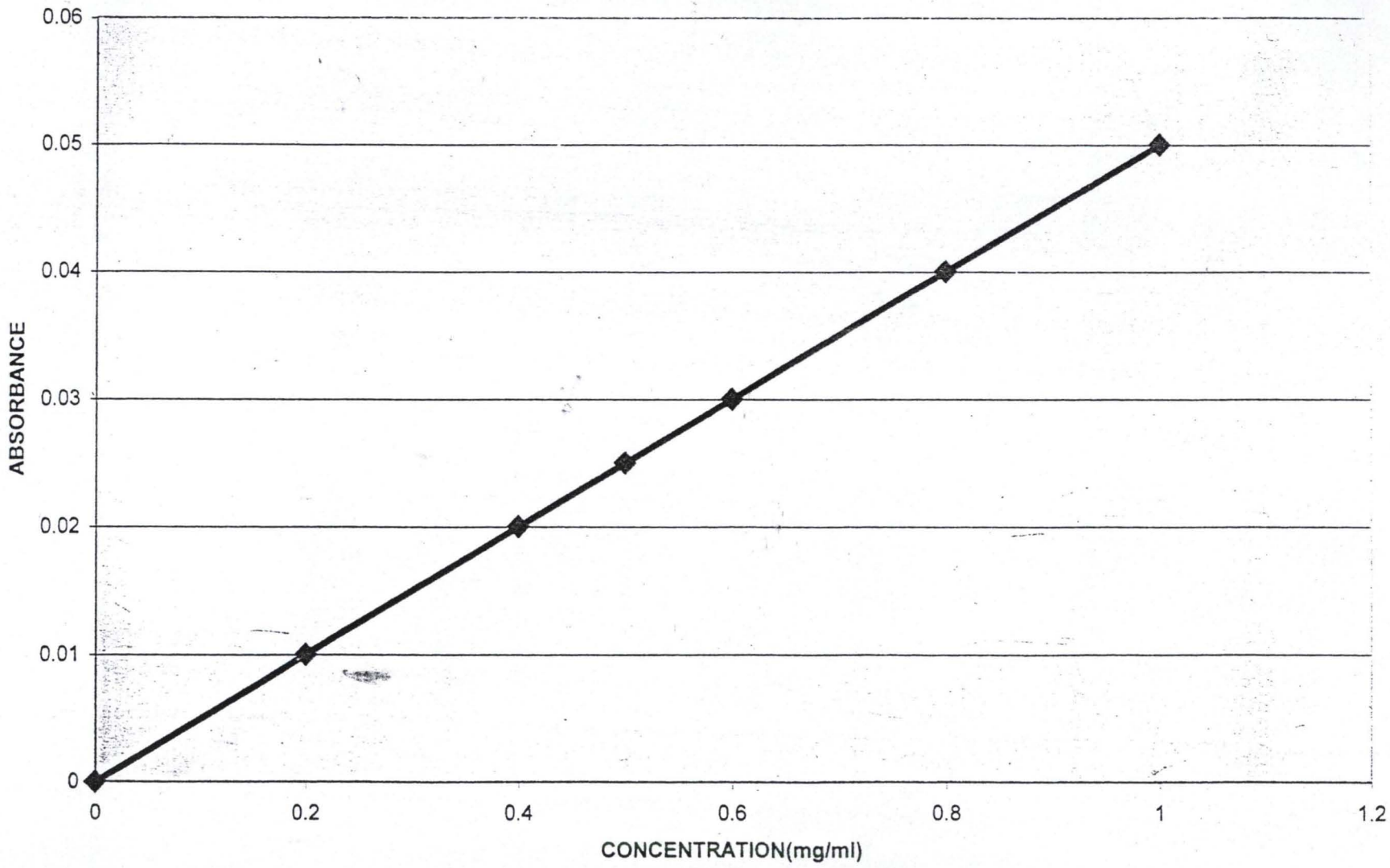
Conc. Of Test = Absorbency of test (AT) x Conc. Of standard

Absorbence of standard (AS)

$$CT = \frac{AT}{AS} \times CS$$

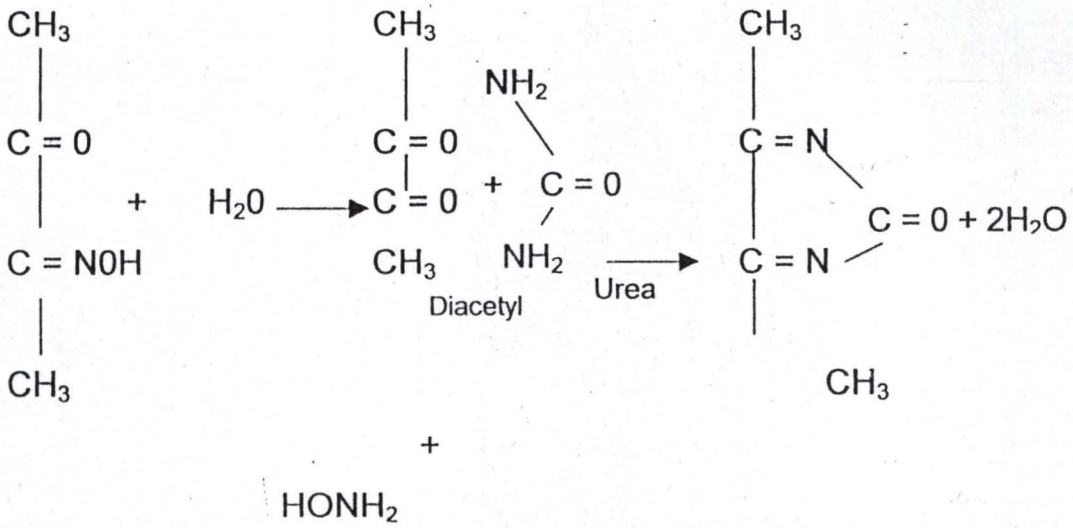
APPENDIX 1C

CALIBRATON CURVE FOR BOVINE SERUM ALBUMIN



APPENDIX IIA THE PRINCIPLE OF DIACETYL MONOXINE FOR THE SERUM UREA DETERMINATION

PRINCIPLE:- Urea reacts with diacetyl monoxine at high temperature in an acidic medium in the presence of cadmium ion and the semi carbazide, to give a red coloured compound which could be measured in colorimeter using a green filter or spectrophotometer at a wavelength of 530nm.



Diacetyl Monoxine Hydroxyl amine Diazine Derivative

APPENDIX IIB THE PRINCIPLE OF Alkaline picrate–slot method FOR THE DETERMINATION OF SERUM CREATININE

Principle: - In slot method, creatinine react with picric acid in alkaline medium, producing a yellow–red compound, which could be measured in a colorimeter using a blue – green filter or in a spectrophotometer at a wavelength of 490nm.

APPENDIX IIC THE PRINCIPLE OF Flame photometry method FOR THE DETERMINATION OF SERUM SODIUM AND POTASSIUM IONS (Na^+ & K^+).

PRINCIPLE: Potassium or sodium solution when sprayed as a fine mist of droplets (nebulised) in to a non-luminous gas flaming, the characteristic emission of the potassium or sodium metallic ions in the samples. the light of a wave length corresponding to the metal (770nm for K^+ and 590nm for Na^+) is selected by a light filter or prism system. The light is allowed to fall on a photosensitive detector system, which measures the quantity of light emitted depending on the concentration of the metallic ions present.

APPENDIX IID THE PRINCIPLE OF TITRIMETRIC METHOD FOR THE DETERMINATION OF SERUM BICARBONATE (HCO_3^-)

PRINCIPLE: A known amount of strong tetraoxosulphate (vi) acid (H_2SO_4) was added to sample of serum or plasma. The carbon(iv)oxide formed was expelled by shaking the preparation. The hydrogen ions that remain are titrated against sodium hydroxide (NaOH) using phenol red as indicator.

APPENDIX IIIA
MALE (CHILDREN) PATIENTS RESULT MPS (+)

S/No	SEX	AGE	MPS	PROTEIN mg/dl	UREA mM/L	CREAT µM/L	Na^+ mM/L	K^+ mM/L	HCO^-_3 mM/L	Cl^- mM/L
026	M	12yrs	+	38.8	2.5	69	134	4.3	38	100
041	M	4yrs	+	48.0	2.0	79	152	4.9	25	120
047	M	8yrs	+	11.0	8.2	120	130	5.7	31	110
159	M	12yrs	+	8.0	2.2	88	125	3.8	26	105
164	M	10yrs	+	22.0	2.2	74	130	5.0	24	107
205	M	10yrs	+	8.0	8.4	130	140	4.5	24	106

MALE (CHILDREN) PATIENT RESULT MPS (++)

/NO	SEX	AGE	MPS	PROTEIN mg/dl	UREA ,mM/L	CREAT. mM/L	Na^+ mM/L	K^+ mM/L	HCO^-_3 mM/L	Cl^- mM/L
24	M	7yrs	++	4.0	2.6	75	134	5.0	32	106
28	M	14yrs	++	30.0	2.4	72	136	5.6	32	106
46	M	14yrs	++	68.4	4.2	100	142	4.8	30	102
56	M	13yrs	++	39.0	2.9	89	120	3.5	27	106
80	M	10yrs	++	8.0	9.0	175	130	3.5	26	107
35	M	13yrs	++	8.0	4.8	108	140	3.8	25	115
24	M	12yrs	++	15.0	6.5	100	125	4.0	30	-
03	M	10yrs	++	26.0	4.5	110	122	3.5	30	105
09	M	9yrs	++	42.0	2.6	80	125	3.0	26	105
10	M	12yrs	++	22.0	3.6	100	135	3.2	26	103
62	M	12yrs	++	26.0	10.3	163	120	3.5	24	86

MALE (CHILDREN) PATIENT RESULT MPS (+++)

S/NO	SEX	AGE	MPS	PROTEIN mg/dl	UREA mM/L	CREAT. µM/L	Na^+ mM/L	K^+ mM/L	HCO^-_3 mM/L	ClmM/L
208	M	11yrs	+++	22.0	7.0	130	130	4.0	30	100

APPENDIX IIIB
MALE (ADULT) PATIENT RESULT MPS (+)

S/NO	SEX	AGE	MPS	PROTEIN mg/dl	UREA mM/L	CREAT. mM/L	Na^+ mM/L	K^+ mM/L	HCO^-_3 mM/L	Cl^- mM/L
036	M	30yrs	+	28.0	2.6	73	140	3.3	28	100
037	M	27yrs	+	36.0	2.3	77	122.	3.6	25	106
007	M	58yrs	+	18.8	3.6	105	100.	4.5	-	-
008	M	26yrs	+	54.0	3.3	80	125.	4.0	26	120
043	M	31yrs	+	54.0	3.7	78	120.	2.6	24	90
005	M	45yrs	+	20.0	3.5	108	130	4.0	25	108
048	M	20yrs	+	60.0	3.4	101	134.	4.3	27	101
064	M	25yrs	+	15.0	1.3	105.2	125.	3.0	30	100
066	M	30yrs	+	20.0	4.3	160	130.	3.6	26	104
072	M	30yrs	+	25.0	3.8	112.6	125.	3.6	26	102
073	M	20yrs	+	22.0	5.6	110	125.	4.5	26	98
089	M	34yrs	+	23.0	3.8	66	130.	4.9	26	100
101	M	23yrs	+	38.0	4.6	121	138	3.0	25	108
107	M	36yrs	+	22.8	3.6	85	120.	4.0	25	98
111	M	2(yrs	+	16.0	3.5	133	140	4.0	30	88
132	M	30yrs	+	16.0	4.5	105	140	4.0	21	120
139	M	22yrs	+	52.0	10.1	186.6	138	3.8	25	110
140	M	25yrs	+	21	4.0	105	140	4.0	26	107
141	M	35yrs	+	20	4.0	127.6	120	3.5	25	98
144	M	29yrs	+	18.0	3.7	102	120	4.0	28	100
161	M	24yrs	+	18.0	3.5	95	130	3.5	27	106
166	M	25yrs	+	11.0	3.7	97	140	5.0	28	108
170	M	25yrs	+	12.0	7.1	140	125	3.5	25	106
171	M	25yrs	+	17.4	6.5	130	130	4.0	25	106
172	M	21yrs	+	18.8	18.5	235	135	3.8	25	107
175	M	45yrs	+	34	8.1	160	130	3.8	26	106
178	M	25yrs	+	13.0	9.5	152	135	3.8	27	107
186	M	25yrs	+	10.0	8.5	168.1	120	4.5	23	108
188	M	22yrs	+	22.0	6.4	120.1	130	3.8	24	105
191	M	21yrs	+	16.0	5.7	147.1	120	4.5	24	106
195	M	21yrs	+	8.0	3.5	98	125	3.0	25	106
196	M	35yrs	+	6.0	6.1	112	135	3.8	25	105
198	M	22yrs	+	7.0	9.3	169	138	4.5	28	105
206	M	17yrs	+	6.2	4.6	105	140	4.8	25	106

APPENDIX IIIC
MALE (ADULT) PATIENT RESULT (MP++)

S/NO	SEX	AGE	MPS	PROTEIN Mg/dl	UREA mM/L	CREAT µM/L	NA^+ mM/L	K^+ mM/L	HCO^-_3 mM/L	Cl^- mM/L
003	M	33yrs	++	18.8	3.9	116.3	140	4.9	25	118
016	M	30yrs	++	40.0	3.1	105	140	5.0	26	111
018	M	30yrs	++	20.0	2.8	77	133	5.2	28	108
022	M	22yrs	++	20.0	2.9	79	138	4.7	32	105
027	M	37yrs	++	34.0	3.0	74	139	3.7	27	101
030	M	28yrs	++	22.0	4.0	79	130	5.5	31	90
031	M	16yrs	++	26.8	2.3	61	136	4.9	36	102
034	M	42yrs	++	18.2	2.6	73	140	3.3	28	100
042	M	16yrs	++	59.0	1.5	62	130	5.2	21	119
011	M	45yrs	++	26.8	7.3	161	130	4.0	26	120
044	M	31yrs	++	68.4	4.2	95	135	3.1	26	101
067	M	20yrs	++	15.0	4.5	115.3	135	4.0	26	111
070	M	22yrs	++	26.0	1.9	118.5	120	5.0	25	106
074	M	26yrs	++	15.0	6.4	114.6	130	5.0	28	112
097	M	22yrs	++	47.6	3.6	103	138	3.6	25	117
102	M	19yrs	++	48.0	3.5	85	120	4.0	25	100
108	M	25yrs	++	34.0	6.5	113	144	3.6	23	102
110	M	56yrs	++	34.0	5.4	107	140	4.0	26	108
112	M	53yrs	++	11.0	3.0	100	142	3.0	27	-
114	M	22yrs	++	28.0	3.0	100	136	3.3	25	104
124	M	18yrs	++	15.0	6.5	100	125	4.0	21	120
137	M	30yrs	++	16.0	4.4	101.5	140	3.7	25	102
142	M	21yrs	++	11.4	2.7	86	120	3.8	30	110
145	M	19yrs	++	17.0	4.5	116	120	4.2	25	100
150	M	50yrs	++	11.0	4.9	109	136	4.0	25	100
151	M	26yrs	++	9.4	4.8	106	140	4.0	25	107
154	M	20yrs	++	7.4	4.6	105	130	3.8	25	106
157	M	27yrs	++	13.5	3.7	94	120	3.6	26	105
158	M	27yrs	++	8.0	3.2	98	130	3.8	28	107
163	M	22yrs	++	28.0	3.9	104	130	4.0	26	108
167	M	32yrs	++	11.4	3.7	98	130	4.5	26	100

169	M	19yrs	++	16.0	10.5	209	130	3.6	27	107
173	M	26yrs	++	35.0	18.1	229	130	3.8	27	107
176	M	20yrs	++	35.6	3.5	105	135	4.2	27	108
183	M	26yrs	++	30.0	10.2	196	130	4.0	25	105
185	M	50yrs	++	26.0	7.6	145	120	4.0	22	98
187	M	21yrs	++	26.0	5.7	140	130	4.0	24	108
189	M	21yrs	++	18.0	4.1	107	125	4.5	21	106
199	M	20yrs	++	16.0	8.7	146	130	3.7	28	106
200	M	20yrs	++	72.0	11.4	190	136	4.0	26	107
202	M	30yrs	++	15.0	7.50	140	130	4.5	25	108
160	M	32yrs	++	16.0	15.5	206	138	3.8	26	106
212	M	15yrs	++	8.0	5.7	118	138	3.8	26	106

APPENDIX IIID

MALE ADULTS PATIENT RESULT MPS (+++)

029	M	25yrs	+++	260.0	1.2	65	140	7.0	21	86
019	M	31yrs	+++	54.0	4.2	118	130	3.5	26	100
093	M	27yrs	+++	15.0	5.8	111	134	4.0	25	108
120	M	25yrs	+++	13.5	5.5	95	130	4.0	28	100
121	M	65yrs	+++	12.8	2.8	70	126	3.6	26	90
201	M	25yrs	+++	2.0	6.3	100	125	3.8	30	108

MALE ADULTS PATIENT RESULT MPS (NUMEROUS)

184	M	20yrs	NUM.	3.0	4.5	115.2	122	3.8	106	26
192	M	22yrs	NUM.	42.0	5.9	108	120	3.5	102	25

APPENDIX IVA
FEMALE CHILDREN (PATIENTS) MPS (+)

S/No	SEX	AGE	MPS	PROTEIN mg/dl	UREA mM/L	CREAT. µM/L	Na^+ mM/L	K^+ mM/L	HCO_3^- mM/L	Cl^- mM/L
094	F	10yrs	+	27.4	4.1	105	135	3.5	28	108
105	F	12yrs	+	86.0	7.5.	163	128	4.0	25	108
116	F	2yrs	+	28.0	3.5	-	130	4.2	22	94
168	F	13yrs	+	22.0	14.7.	241	125	3.5	26	108
204	F	11yrs	+	22.0	8.4	130	138	4.0	28	105

MPS (++)

103	F	6yrs	++	47.0	2.2	79	134	4.0	27	98
123	F	14yrs	++	13.5	3.2	118	140	3.5	24	84
006	F	10yrs	++	26.8	3.1	100	126	4.0	27	118
014	F	10yrs	++	26.8	3.0	109	138	4.4	30	112
193	F	9yrs	++	48.0	7.8	149	130	5.0	26	107
194	F	6yrs	++	6.0	4.7	107	140	4.2	26	105
213	F	14yrs	++	20.0	7.0	140	128	3.5	27	107

MPS (+++)

109	F	9yrs	+++	44.8	2.0	90	140	3.4	30	84
021	F	10yrs	+++	13.5	5.5	101	138	5.8	34	104
177	F	10yrs	+++	108.0	9.4	160	130	3.8	28	108
207	F	12yrs	+++	44.0	3.8	125	125	4.2	26	110
211	F	13yrs	+++	12.0	4.3	116	137	3.8	27	104

APPENDIX IVB
FEMALE ADULT NON PREG(PATIENT) MPS (+) RESULT

		AGE	MPS	PROTEIN mg/dl	UREA mM/L	CREAT µM/L	Na^+ mM/L	K^+ Mm/L	HCO^-_3 mM/L	Cl^- mM/L
032	F	39yrs	+	73.0	2.6	66	140	6.0	27	107
020	F	26yrs	+	13.5	2.3	-	138	4.7	26	102
069	F	38yrs	+	12.0	2.8	103.5	130	3.2	31	108
071	F	20yrs	+	25.0	3.4	84.1	120	3.8	22	100
082	F	25yrs	+	21.0	4.3	101	125	4.6	24	104
118	F	31yrs	+	22.0	3.6	104	122	5.0	28	106
128	F	23yrs	+	10.0	2.9	88	128	3.8	28	100
130	F	19yr	+	22.0	8.4	160	120	3.1	24	88
134	F	Adult	+	12.0	4.6	105.9	138	3.6	25	110
138	F	Adult	+	16.0	3.3	104	138	3.8	25	110
146	F	20yrs	+	17.4	4.2	110	120	4.5	24	98
153	F	45yrs	+	11.0	5.4	118	140	5.0	26	107
179	F	45yrs	+	10.0	5.4	120	138	3.8	27	107
181	F	Adult	+	10.0	8.2	160	130	4.0	26	106
182	F	Adult	+	5.0	7.0	140	140	3.8	28	108
190	F	50yrs	+	13.0	10.6	198	122	4.5	26	103
206	F	17yrs	+	6.2	4.6	105	140	4.8	25	106

Appendix IVC
FEMALE (PATIENTS) ADULT NON-PREG (MPS (++)) RESULT

S/NO	SEX	AGE	MPS	PROTEIN mg/dl	UREA mM/L	CREAT. µM/L	Na^+ mM/L	K^+ mM/L	HCO^-_3 mM/L	Cl^- mM/L
)13	F	18yrs	++	40.0.	3.8	98	134	5.2-	31-	105
)19	F	37yrs	++	26.0.	3.0	80	132	4.5-	26-	105
)23	F	45yrs	++	47.0.	4.0	82	142	5.8-	37-	110
)25	F	31yrs	++	22.0.	2.4	68	152	52-	32-	106
)33	F	30yrs	++	3.4.	3.3	77	120	4.7-	30-	113
)35	F	18yrs	++	31.0	2.1	74	138	3.7-	30-	108-
)40	F	17yrs	++	31.0	2.1	69	138	5.6-	32-	101-
)09	F	30yrs	++	26.8	3.6	107	140	4.7	27-	101
)45	F	24yrs	++	36.0	5.3	97	120	5.	27-	89-
)49	F	33yrs	++	64.0	2.7	88	140	3.8	25-	99-
)68	F	19yrs	++	14.0	4.0	105	126	4.5	24-	144-
)80	F	27yrs	++	23.0	3.5	81	130	5.0	27-	107-
)88	F	35yrs	++	14.8	2.5	74	130	3.8	28-	103-
)90	F	43yrs	++	28.8	4.8	86	130	4.3	25-	108-
)99	F	19yrs	++	26.0	115	112	140	3.5	28-	118-
115	F	22yrs	++	62.0	2.9	121	138	3.8	23-	80-
129	F	21yrs	++	45.0	5.0	115	130	3.5	32-	108-
131	F	20yrs	++	18.0	6.2	106	140	4.0	28-	110-
148	F	19yrs	++	8.0	3.9	100	125	4.0	28-	96-
149	F	25yrs	++	18.8	3.2	92	138	4.0	26-	107-
168	F	35yrs	++	14.8	2.5	74	130	3.8	28-	103-

APPENDIX IVD
FEMALE (ADULT) NON PREG PATIENTS MPS (+++)

S/NO	SEX	AGE	MPS	PROTEIN mg/dl	UREA mM/L	CREAT. µM/L	Na^+ mM/L	K^+ mM/L	HCO_3^- mM/L	Cl^- mM/L
015	F	26yrs	+++	26.8	3.2	111	140	4.5	32	100
017	F	32yrs	+++	26.8	2.5	76	135	4.7	30	107
092	F	18yrs	+++	31.0	2.0	76	140	3.3	28	107
100	F	35yrs	+++	13.5	2.3	108	135	3.6	24	107
136	F	Adult	+++	42.0	5.6	127	138	4.0	26	100
143	F	20yrs	+++	17.4	4.7	115	125	5.0	26	100
152	F	26yrs	+++	12.8	2.8	96	138	3.5	25	106
155	F	23yrs	+++	9.4	4.5	117	130	3.8	25	107
162	F	25yrs	+++	12.8	3.1	78	138	4.0	24	108
197	F	22yrs	+++	5.6	6.5	106	130	3.5	24	110
165	F	19yrs	+++	11.0	2.3	130	5.0	24	107	49

APPENDIX IVE

FEMALE ADULT PREGNANT(PATIENT) MPS (+)

		AGE	MPS	PROTEIN Mg/dl	UREA Mmol/l	CREAT Mmol/l	Na^+ Mmol/l	K^+ Mmol/l	HCO^-_3 Mmol/l	Cl^- Mmol/l
038	F	23yrs	+	28.0	1.0	60	140	5.6	28	106
039	F	20yrs	+	28.0	1.4	67	140	4.4	26	100
065	F	34yrs	+	44.0	3.8	117.9	130	3.6	28	104
078	F	30yrs	+	15.0	3.6	80.5	126	4.1	26	102
083	F	25yrs	+	42.0	4.3	98	125	4.6	26	115
085	F	21yrs	+	14.0	6.9	113	140	3.8	24	106
087	F	19yrs	+	14.0	2.7	86	133	4.2	24	105
104	F	25yrs	+	40.0	1.7	97	140	3.8	21	106
106	F	25yrs	+	32.0	2.9	85	132	3.5	30	110
122	F	23yrs	+	27.0	2.7	73	125	3.5	30	89
126	F	26yrs	+	9.4	3.0	142	140	4.2	26	100
147	F	28yrs	+	15.0	2.8	111	120	3.8	25	108
001	F	27yrs	+	24.0	7.3	179.1	139	4.8	29	115
002	F	28yrs	+	13.5	4.0	148.8	144	5.0	23	121
004	F	50yrs	+	20.0	4.0	135.4	139	4.8	23	115

(MP++)

012	F	25yrs	++	20.0	3.7	100	136	4.3	29-	110-
075	F	28yrs	++	14.8	7.9	111	130	5.1	30-	106-
077	F	28yrs	++	20.0	6.2	131.3	128	4.5	27-	115-
079	F	22yrs	++	15.0	3.3	118.2	128	3.4	24-	104-
081	F	20yrs	++	13.5	3.9	103	135	4.8	24-	116-
084	F	23yrs	++	11.3	3.9	106	130	3.8	30-	120-
086	F	34yrs	++	30.0	3.1	125	130	5.5	26-	100-
091	F	20yrs	++	18.2	3.0	81	135	3.0	26-	107-
098	F	23yrs	++	44.8	1.5	79	130	4.0	24-	108-
113	F	28yrs	++	15.0	3.6	83	140	4.2	25-	-
063	F	35yrs	++	16.0	3.1	91	138	3.6	24-	105-

(MP+++)

017	F	82yrs	+++	26.8	2.5	76	135	4.7	3.0	107
076	F	29yrs	+++	11.3	3.1	95	130	3.2	28	110
095	F	27yrs	+++	55.0	3.1	84	140	3.0	24	107
096	F	25yrs	+++	50.0	3.2	98	140	3.5	25	116
117	F	16yrs	+++	31.0	3.1	83	125	5.0	26	106
127	F	20yrs	+++	22.0	2.6	113	136	3.8	24	96
174	F	31yrs	+++	32.0	5.0	107	131	4.0	26	102

APPENDIX VA
RESULT OF MALE NON-MALARIAL INDIVIDUALS

S/NO	SEX	AGE	PROTEIN Mg/dl	UREA mM/L	CREAT µM/L	NA^+ mM/L	K^+ mM/L	HCO_3^- mM/L	Cl^- mM/L
C05	M	12yrs	13.4	3.2	107	138	5.0	28	100
C 05	M	11yrs	12.0	2.5	99	129	4.5	32	105
C 07	M	8yrs	11.0	4.3	98	136	4.6	28	106
C 01	M	32yrs	10.8	4.2	61	124	5.0	25	108
C 02	M	26yrs	15.4	2.9	89	126	3.6	24	106
C 03	M	30yrs	14.0	4.6	104	128	4.4	26	105
C 04	M	16yrs	13.4	4.8	88	120	4.3	25	128
C10	M	22yrs	16.0	5.8	105	120	3.5	28	108
C 11	M	21yrs	9.4	8.4	150	120	3.1	24	88
C 12	M	23yrs	14.0	8.3	148	125	4.0	25	105
C14	M	29yrs	4.0	4.5	110	125	3.7	25	104
C15	M	19yrs	5.0	5.8	107	125	3.6	24	106
C16	M	23yrs	12.0	4.5	98	130	3.5	28	105
C17	M	28yrs	6.0	5.6	116	120	4.4	27	108
C18	M	20yrs	18.0	7.1	160	122	4.8	28	110
C19	M	19yrs	8.0	6.0	103	140	5.0	22	104
C20	M	20yrs	16.0	4.5	107	125	5.0	25	106
C22	M	20yrs	16.0	4.5	107	125	5.0	25	106
C25	M	22yrs	14.0	3.5	96	125	3.6	26	105
C23	M	22yrs	5.0	3.5	98	140	4.5	20	108
C27	M	20yrs	10.0	4.5	103	125	3.6	23	100
C31	M	22yrs	9.0	3.8	106	132	4.5	24	114
C33	M	22yrs	9.0	6.2	110	125	4.0	28	100
C35	M	22yrs	12.0	5.4	106	136	4.5	27	108
C36	M	22yrs	16.0	4.5	100	120	4.0	27	107
C41	M	19yrs	12.0	7.9	125	130	4.0	28	106
C42	M	20yrs	18.0	4.4	109	125	4.5	30	105
C43	M	24yrs	17.0	3.5	102	125	3.8	29	108
C44	M	23yrs	10.0	4.3	106	125	4.5	30	100

C42	M	20yrs	18.0	4.4	109	125	4.5	30	105
C43	M	24yrs	17.0	3.5	102	125	3.8	29	108
C44	M	23yrs	10.0	4.3	106	125	4.5	30	100
C45	M	18yrs	4.0	4.2	108	125	4.5	31	110
C46	M	35yrs	12.0	5.3	110	130	5.0	30	90
C47	M	27trs	6.0	4.4	118	125	4.5	28	106
C48	M	20yrs	8.0	3.8	104	140	4.5	25	108
C49	M	22yrs	26.0	4.4	151	130	5.0	27	107
C50	M	23yrs	15.0	8.4	140	138	4.0	26	106
C28	M	22yrs	8.0	3.7	102	140	5.0	24	116
C57	M	27yrs	13.4	5.4	88	136	4.6	30	111
C51	M	18yrs	20.0	4.4	98	130	4.5	25	106
C52	M	23yrs	14.0	4.2	106	140	5.0	24	106
C53	M	23yrs	12.0	4.2	109	138	4.5	25	106
C54	M	22yrs	6.0	4.8	112	130	4.2	27	106
C55	M	22yrs	8.0	5.1	101	140	5.0	24	107
C58	M	27yrs	13.0	5.4	88	136	4.6	30	111