بسم الله الرحمن الرحيم

**Shendi University**
College of Graduate Studies &Scientific Research

# Renal and Liver Function Tests in Association with Some Haematological Parameters in Malaria Ambulant patients in Shendi locality, River Nile State, SUDAN.

A thesis Submitted for
**PhD** degree in Clinical Chemistry

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# الآيـة

قال الله تعالي:

***بسم الله الرحمن الرحيم***

﴿ فَتَعَالَى اللَّهُ الْمَلِكُ الْحَقُّ وَلَا تَعْجَلْ بِالْقُرْآنِ مِن قَبْلِ أَن يُقْضَى إِلَيْكَ وَحْيُهُ وَقُل رَّبِّ زِدْنِي عِلْماً ﴾

**صدق الله العظيم**

**سورة طه الآيـة 114**

# Dedeication

*To Soul of my mother…….*

*To Soul of my father……..*

*To my Wife …..*

*To my kids (Ibrahim & Limar)…..*

*To my brother……..*

*To my sisters……*

*I dedicate this work with my best wishes to all.*

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# *List of abbreviations*

| Abbreviations | Meaning |
|---|---|
| **ABER** | Annual Blood Examination Rate |
| **ACE** | Angiotensin Converting Enzyme |
| **ADH** | Anti Diuretic Hormone |
| **AIDS** | Acquired Immuno Deficiency Syndrome |
| **ALP** | Alkaline phosphatase |
| **ALT** | Alanine amino Transferase |
| **API** | Annual Parasitic Incidence |
| **ARB:** | Angiotensin Receptor Blocker |
| **ARF** | Acute Renal Failure |
| **AST** | Aspartate Aminotransferase |
| **ATP** | Adenosine Triphosphate |
| **BBB** | Blood Brain Barrier |
| **BC** | Before Christmas |
| **BUN** | Blood Urea Nitrogen |
| **CAP** | College of American Pathologists |
| **CKD** | Chronic Kidney Disease |
| **Cr Cl** | Creatinine Clearance |
| **CSA** | Chondroitin Sulfate A |
| **CV** | Coefficient of Variation |
| **DARC** | Duffy Antigen Receptor for chemokines |
| **DBL- EBP** | Duffy Binding like- Erythrocyte Binding Protein |
| **DBLP** | Duffy Binding like Protein |
| **DCs** | Denderitic Cells |

| | |
|---|---|
| **DIC** | Disseminated Intravascular Coagulation |
| **DM** | Diabetes Mellitus |
| **EIA** | Enzyme Immuno Assay |
| **FN** | False Negative |
| **FP** | False Positive |
| **GFR** | Glomerular Filtration Rate |
| **GGT** | Gamma Glutamyl Transferase |
| **GPI** | Glycosyl Phosphatidyl Inositol |
| **HIV** | Human Immuno Virus |
| **ICAM** | Intracellular Adhesion Molecule |
| **ICT** | Immuno Chromatography Test |
| **IDMS** | Isotope Dilution Mass Spectrometry |
| **IFNɤ** | Interferon Gamma |
| **IGF -1** | Insulin like Growth Factor |
| **IL- 6** | Interleukin -6 |
| **INR** | International Normalization Ratio |
| **ITP** | Immuno Thrombocytopenia |
| **MHC** | Major Histo Compatibility |
| **MI** | Myocardial Infraction |
| **MPV** | Mean Platelet Volume |
| **MSP** | Merozoite Surface Protein |
| **$NADP^+$** | Nicotinamide Adenine Dinucleotide Phosphate |
| **NADPH** | Nicotinamide adenine dinucleotide phosphate hydrogen |
| **NK** | Natural Killer |
| **NO** | Nitric Oxide |
| **PVNR** | Predictive Value for Negative Result |

| | |
|---|---|
| **PCR** | Polymerase Chain Reaction |
| **PDW** | Platelet Distribution Width |
| **PF%** | Plasmodium Falciparum Percentage |
| **PfEMP1** | Plasmodium falciparum Erythrocyte Membrane Protein1 |
| **PLDH** | Plasmodium Lactate Dehydrogenase |
| **PVPR** | Predictive Value for Positive result |
| **PRBC** | Phagocytosis Red Blood Cell |
| **PRR** | Pattern Recognition Receptor |
| **PT** | Prothrombin Time |
| **PV** | Predictive Value |
| **RBM** | Roll Back Malaria |
| **RDTs** | Rabid Diagnostic Tests |
| **SFR** | Slide Falciparum Rate |
| **SGOT** | Serum Glutamate Oxaloacetate Transaminase |
| **SGPT** | Serum Glutamate Pyruvate Transaminase |
| **SIADH** | Syndrome of Inappropriate Anti Diuretic Hormone Secretion |
| **SLE** | Systemic Lupus Erythematosus |
| **SPR** | Slide Positivity Rate |
| **TN** | True Negative |
| **TNF** | Tumor Necrosis Factor |
| **TP** | True Positive |
| **TSRA** | Thrombo Spondin Related Anonymous |
| **TLR** | Toll Like Receptor |
| **UDPGT** | Uridyl Diphosphate Glucuronyl Transferase |
| **WHO** | World Health Organization |

# ملخص الدراسه


أجريت دراسة توقعيه لاختبارات وظائف الكلي والكبد وتعداد الصفائح الدمويه ومعاملاتها في 153 مريض سائر مصاب بداء البرداء الحاد من المراكز الصحيه وبعض المستشفيات بمحلية شندي ولايه نهر النيل في الفتره من نوفمبر 2010 وحتي اكتوبر 2014.

من بين هؤلاء 92 من الذكور(60,1%) و58 من الاناث (39,9%) تراوحت اعمارهم بين 12 – 65 عاما بوسط حسابي 31,3 عاما.

مائه واثنين وعشرون من هؤلاء المرضي مصابون بالمتصوره المنجليه (79,9%) بينما واحد وثلاثون مصابون بالمتصوره النشطه .

شملت الدراسه خمسين من المعادلين, واحد وثلاثون منهم (62%) من الذكور وتسعة عشر من الاناث (38%) تراوحت اعمارهم بين 16 – 61 عاما بوسط حسابي 32,4 عاما.

أظهر فحص البول وجود بيله بروتينيه في 9 مرضي مصابون بالحمي. زادت نسبه حدوث البيله البروتينيه بزيادة درجه الطفيليه .

وجدت الاسطوانات الحبيبيه في بول 75 مريضا وهي من النوع الصغير ضيقه التجويف. لاتوجد علاقه احصائيه بين هذه الاسطوانات الحبيبيه ونوع المتصوره او درجه الطفيليه او عمر المرضي.

أظهرت الدراسه بان الوسط الحسابي لمادتي البولينا والكرياتينين في مصل المرضي يزيد عن الوسط الحسابي في المعادلين مع وجود علاقه احصائيه بينهما (القيمه الاحتماليه اقل من 0.05)

معدل مادة الصوديوم في مصل المرضي اقل عنه في مصل المعادلين وهو فرق ذودلاله احصا ئيه (القيمه الاحتماليه اقل من 0.05).

متوسط تركيز البوتاسيوم في مصل المرضي والمعادلين لم يظهر اختلافا ذو دلاله احصائيه (القيمه الاحتماليه اكبر من 0.05).

في 15 مريض ظهرت علامات انحلال الدم والتي ظهرت في زياده مولد اليوروبيلين في بول المرضي وارتفاع معدل البيليروبين غير المباشر (0.8 – 3,8 ملغرام/دل)

أظهرت الدراسه وجود علاقه احصائيه بين زياده مده الأعراض عند المرضي والزياده في معدل خميره غلوتاميت الاوكسالواستات ترانساميناز في المصل.

بينت الدراسه وجود انخفاض قليل في مستوي الالبيومين في مصل المرضي مقارنه بمعدله في المعادلين ولكن بدلاله احصائيه (القيمه الاحتماليه اقل من 0.05).

اظهرت الدراسه فرقا ذو دلاله احصائيه تمثل في زياده مستوي خميره الاسبارتيت ترانساميناز والالنين ترانساميناز في مصل المرضي عنه في مصل المعادلين (القيمه الاحتماليه اقل من 0.05).

لم تظهر اي علاقه احصائيه بين البروتين الكلي, خميره الفوسفاتيز القلويه وخميره الغاما غلوتامايل ترانسفيريز في مصل كل من المرضي والمعادلين (القيمه الاحتماليه اكبر من 0.05).

# Abstract

A prospective study of renal, liver function tests and platelets count in (153) ambulant patients with severe malaria from health centers and some hospitals in Shendi locality River Nile State in the period from November 2010 until October 2014. Among these 92 males (60.1%) and 58 females (39.9%), their ages ranged from (12-65) years with a mean of (31.3) years.

One hundred and twenty-two of these patients are infected with P. falciparum (79.9%), while thirty-one are infected with *P. vivax.*

Fifty of controls were included in the study, thirty-one of them (62%) were males and nineteen females (38%), their age ranged from (16-61) years, mean (32.4) years.

Urine examination showed the presence of proteinuria in (9) febrile patients. The incidence of proteinuria was directly correlated with degree of parasitaemia.

Narrow bore granular cast were found in (75) patients and were not statistically correlated with plasmodium species, degree of parasitaemia or age of the patients.

The study showed that the means of both Blood urea and serum creatinine were elevated in patients compared to those of controls with a statistically significant difference *(p-value <0.05).*

Mean sodium concentration in the serum of patients was found to be lower than in serum of controls with statistically significant difference. *(P.ovale* < 0.05).

Average potassium concentration in the serum of patients and controls showed no statistically significant difference *(p-value > 0.05).*

Fifteen patients showed signs of hemolysis, which detected by increased urinary urobilinogen and elevated serum indirect bilirubin (0.8 - 3.8 mg / dl).

The study showed a statistically significant direct correlation between duration of symptoms and the increasing in level of *SGOT*, (recently called AST)

The study showed a slightly decrease in the level of albumin in the serum of patients compared with controls, and the difference was statistically significant *(p-value < 0.05).*

The study showed a statistically significant difference between patients and control in serum *SGOT* level (*p-value <0.05).*

Serum total protein, alkaline phosphatase and gamma glutamyl transferase showed no statistically significant difference between patients and controls. *(P-value greater than 0.05).*

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# 1.1: Introduction

Malaria is a mosquito-borne infectious disease of humans and other animals caused by parasitic protozoans (a type of unicellular microorganism) of the genus *Plasmodium*. Commonly, the disease is transmitted by a bite from an infected female Anopheles mosquito, which introduces the organisms from its saliva into a person's circulatory system. In the blood, the parasites travel to the liver to mature and reproduce [(1)]. Five species of *Plasmodium* can infect humans. The vast majority of deaths are caused by *Plasmodium falciparum* and *Plasmodium vivax*, while *Plasmodium ovale*, and *Plasmodium malariae* cause a generally milder form of malaria that is rarely fatal. The zoonotic species *Plasmodium knowlesi,* prevalent in Southeast Asia, causes malaria in *macaques* but can also cause severe infections in humans. Malaria is common in tropical and subtropical regions because rainfall, warm temperatures, and stagnant waters provide an environment ideal for mosquito larvae [(2)]. Malaria can be diagnosed clinically and is typically diagnosed by the microscopic examination of blood using blood films, or with antigen-based rapid diagnostic tests. Modern techniques that use the polymerase chain reaction to detect the parasite's *DNA* have also been developed, but these are not widely used in malaria-endemic areas due to their cost and complexity [(3).]The disease is widespread in tropical and subtropical regions in a broad band around the *equator,* including much of Sub-Saharan Africa, Asia, and the Americas. The World Health Organization estimates that in 2010, there were (219) million documented cases of malaria, that year, the disease killed between (660,000) and (1.2) million people, many of whom were children in Africa. [(4)]

According to estimates from the World Health Organization, over (207) million cases and about (627,000) deaths were occurred in 2012. *Plasmodium falciparum* and *P. vivax* are the most common. *P. falciparum* is the most deadly. [(5)]

According to reports of Ministry of health, River Nile state, there were (2865) cases and about 8 deaths were occurred in 2013[6].

In this study, Blood urea, serum creatinine and serum electrolytes has been measured in malaria patients as a biochemical test to evaluate the kidney function, however some studies have found reversible kidney dysfunction. Also, plasma total protein, albumin, total bilirubin, direct & indirect bilirubin and liver enzymes *SGOT, SGPT, ALP* and *GGT* were estimated in patients to assess the liver function during the illness.

Changes in platelet counts during acute malaria are commonly reported in the medical literature, especially in *P. falciparum* infections; such changes are a major cause of concern to clinicians because such cases are more likely to evolve into serious and complicated disease cases [6, 7]. However, many recent studies have also found thrombocytopaenia associated with *P. vivax* [8-9].

## 1.2: Rationale

Malaria is an important cause of death and illness, especially in tropical countries [10]. The World Health Organization reported that malaria is responsible for nearly (90) % of deaths in Africa [11], and it established criteria for severe malaria that assisted clinical and epidemiological studies. This project was begun in 1990, and was then revised in 2000 to include other clinical manifestations and laboratory values that portend a poor prognosis based on clinical experience in semi-immune patients. Several studies documented an increase in malaria incidence as a consequence of irrigation and agricultural development in many countries including Sudan [12]. Epidaemiological patterns of malaria are widely different; such information can help programs design improved strategic interventions. Malaria causes nearly (7.5-10) million cases and (35,000) deaths every year in Sudan [13]

Several study have indicated that sever malaria is a risk factor for acute renal failure (*ARF*). Information on kidney and liver involvement in malaria in Africa is still very scanty.

No study in Shendi locality has specifically assessed the effect of malaria on renal and liver function.

The detection of abnormal finding in those patients will aid or help physician in early prevention of liver, renal disease and fatal haematological changes.

## 1.3: Objectives

### *1.3.1: General objective:*

To evaluate the effect of malaria on selected parameters of liver, renal function and some haematological parameters in ambulant patients with malaria.

### *1.3.2: Specific objectives:*

1. To determine the biochemical parameters of liver function in case & control group.
2. To estimate the biochemical parameters of Renal function in case & control group.
3. To compare the laboratory findings of hepatic, renal functions and platelet count in acute malaria patients according to the malaria species, degree of parasitaemia and duration of infection.
4. To calculate Sensitivity & Specificity of *ICT* (Ag based) comparing with microscopical method as a gold standard technique for malaria diagnosis.
5. To compare the results obtained in the study with other studies done in Sudan and other countries.

# 2. Literature Review

## *2.1: Historical Background of Malaria:*

Malaria ranks among the three major health and developmental challenges facing most of the poorest countries in the tropical and sub-tropical regions of the world. As early as 2700 BC, a disease marked by high fever and an enlarged spleen was noted in ancient China as reported in ancient Chinese documents. Believing the disease was transmitted through the air, the sixteenth-century Italians called the disorder malaria (bad or evil air). *P. vivax* stowed away with the English going to Jamestown, while *P.falciparum* rode along with slaves from Africa. (14)

In the United States, malaria had flourished for centuries in the South and in port cities like Boston and New York. During the civil war, armies on both sides of the war had been stationed in the south after sustaining more than (1.2) million cases of malaria. The southern United States continued to be afflicted with millions of cases of malaria each year into the mid-1930s.

By the 1960s, the *WHO* had scaled its Global Eradication of Malaria Program to one of worldwide malaria control (3). Malaria threatens nearly (40) %of the people in the world (2.4) billion people - mostly in the tropics and subtropics. The disease is currently endemic in (90 to 100) countries. In 1990, (80) % of cases were in Africa, with the remainder found in countries such as India, Brazil, Afghanistan, Sri-Lanka, Thailand, Indonesia, Vietnam, Cambodia and China. *P. falciparum* is the predominant species being responsible for over (120) million new cases and all the malaria deaths per year globally. *P. falciparum* is responsible for the alarming drug-resistant strains now emerging in the most endemic areas. (15)

About (1,200) cases of malaria are diagnosed in the United States each year. Most of these are "imported" by military personnel and travellers to, or

immigrants from, countries where malaria is common. Malaria has occasionally been spread locally in the United States by infected mosquitoes stowed away on international airlines, or, on rare occasions, by mosquitoes there that have bitten someone who was infected. Despite mankind's longstanding struggle to control mosquito populations, the *WHO* currently estimates that each year malaria causes (300) to (500) million infections and (1.5 to 3) million deaths each year. This is an alarming rate given that during the six-month Ebola outbreak in the Democratic Republic of the Congo in 1995, about (250) people died while malaria kills over (5,000) Africans every day [(6)].

The parasite seems to increase greatly one's susceptibility to other infections via generalized immunosuppression. A baby born to a pregnant woman infected with malaria will have a (40) % greater chance of low birth weight, and congenital malaria may account for as many as half of all childhood deaths in Africa [(16)].

In Sudan, malaria accounts for (30-50) % of outpatients in health institutions across the country. (8-10) % of admitted children are due to malaria, with mortality rate of as much as (0.3) million annually, most of them children below the age of (5) years. It therefore becomes very necessary to constantly evaluate the extent of renal and hepatic impairment in malaria cases to ensure proper management of malaria infection with its associated complications. [(17)]

More than (30,000) cases of malaria are reported annually among travelers from developed world visiting malarious areas. With the shrinking globe, perennially prevalent malaria, therefore, remains an ever existing danger for humanity, in every part of the globe. [(18)]

## *2.2: Geographical distribution of malaria in Sudan:*

Based on climate models, it is estimated that (75) % of the population (37) millions are at risk of epidemic malaria. Most of the country below north latitude (150) is endemic zone with high transmission in southern states, while parts of the north are exposed to epidemics following the heavy rains or floods from River Nile. Transmission of malaria in north Sudan south to Khartoum is seasonal and depends on the rains except in urban cities and irrigated schemes. Sudan's rainy season lasts for about three months (July to September) in the north, and up to (6) months (June to November) in the south [(19).] Hence, the duration of the transmission varies from (3-6) months with an average of (4) months, longer season is noticed on the southern areas. The transmission season may last from July/August to November / December, with earlier beginning of in June in the Southern areas (e.g., Kadugli, El Damazin) and later start in August in northern areas. (Wad Medani, Kosti, Kassala, El Obeid) Longer transmission upto (9) months may occur in certain agriculture schemes areas. Urban cities may have another transmission during winter season (December-February ) due to broken water pipes *P. falciparum* is responsible for more than (95) % of malaria cases in Sudan. However, an increase is being reported in malaria cases caused by *P. vivax* (mainly in the eastern part of Sudan bordering Ethiopia and Eretria). [(20)]

## *2.3: Definitions for Malaria Field Work:*

These definitions and indices are important in epidaemiological surveillance and in planning the control strategy for malaria.

**Endemic Malaria:** Constant incidence over a period of many successive years in an area.

**Epidemic Malaria:** Periodic or occasional sharp increase of malaria in a given indigenous community.

**Stable Malaria:** Amount of transmission is high without any marked fluctuation over years though seasonal fluctuations occur.

**Unstable Malaria:** Amount of transmission changes from year to year.

**Vulnerability:** Either proximity to malarious areas or liability to frequent influx of infected people or anophelines.

**Receptivity**: Habitual presence of vector anophelines or existence of ecological factors.

**Clinical Cure:** Relief of symptoms without complete elimination of parasites.

**Radical Cure:** Elimination of parasites actually responsible for attack of malaria.

**Recrudescence:** Renewed clinical activity seen during the first (8-10) weeks after primary attack (short term relapse).

**Recurrence:** Renewed clinical activity seen around ($30^{th}$ - $40^{th}$) week following primary attack (long term relapse)

**Clinically Latent:** Symptom less phase between primary attack and relapse with splenomegaly, no parasite seen in peripheral smear.

**Insecticidal Resistance:** Development of resistance i.e. ability in strains of insects to tolerate doses of toxicants which would prove lethal to the majority of the insect population of the same species.

**Epidemiological Indices:**

**Annual Blood Examination Rate (A.B.E.R.)** = Smears examined in a year (X 100 / Total population).

**Annual Parasitic Incidence (A.P.I.)** = Total no. of positive slides for parasite in a year x 1000 / Total population.

**Annual Falciparum Incidence =** Total positive PF in a year x 1000 / Total population.

**Slide Positivity Rate (S.P.R.)** = Total positive x 100 / Total slides examined.

**Slide Falciparum Rate (S.F.R.)** = Total positive PF x 100 / Slides examined.

**P. falciparum Percentage (PF %)** = Total positive for *P. falciparum* x 100 / Total positive for MP. [21]

## *2.4: Malaria Parasite:*

Malaria is caused by protozoan parasites called *Plasmodia*, belonging to the parasitic phylum Apicomplexa. More than (200) species of the genus Plasmodium (= plasma + eidos, form) have been identified that are parasitic to reptiles, birds, and mammals. [22] Four *Plasmodium species* have been well known to cause human malaria, namely, *P. falciparum, P. vivax, P. ovale*, and *P. malariae*. A fifth one, *P. knowlesi*, has been recently documented to cause human infections in many countries of Southeast Asia. [23]

## *2.5: Transmission of Malaria:*

Laveran, a French army surgeon stationed in Constantine, Algeria, demonstrated the parasites in the blood of a patient suffering from malaria on November 6, 1880, Ronald Ross, a British officer in the Indian Medical Service, solved the problem of malaria transmission by demonstrating the malaria parasite forms (poetically called by him as the million murdering seeds) within the gut wall of the mosquitoes on August 20, 1897.[24,25].

### *2.5.1: Principal mode of transmission:*

Malaria is spread by the bites of female Anopheles mosquito. Of more than (480) species of Anopheles, only about 50 species transmit malaria, with every continent having its own species of these mosquitoes: *An. gambiae* complex in Africa, *An. freeborni* in North America, *An. culicifacies*, *An. fluviatilis*, *An. minimus*, *An. philippinensis*, *An. stephensi*, and *An. sundaicus* in the Indian subcontinent.[26,27] *An. leucosphyrus*, *An. latens*, *An. cracens*, *An. hackeri*, *An. dirus* etc., have been identified as the vectors for the transmission of *P. knowlesi*.[28. 29] The habits of most of the anopheline mosquitoes have been characterized as anthropophilic (prefer human blood meal), endophagic (bite

indoors), and nocturnal (bite at night) with peak biting at midnight, between (11) pm and (2) am.[26] The blood meal from a vertebrate host is essential for the female mosquitoes to nourish their eggs. The mosquitoes find their host by seeking visual, thermal, and olfactory stimuli and of these; carbon dioxide, lactic acid, skin temperature, and moisture are more important mosquito attractants. Depending on the strength of these stimuli, the attractiveness of different persons varies, with adults, men, and larger persons being more attractive than others. [30, 31] When a mosquito bites an infected individual, it sucks the gamaetocytes, the sexual forms of the parasite, along with blood. These gametocytes continue the sexual phase of the cycle within the mosquito gut and the sporozoites that develop then fill the salivary glands of the infested mosquito. When this female mosquito bites another man for a blood meal, the sporozoites are inoculated into the blood stream of the fresh victim, thus spreading the infection.

***Other modes of transmission:***

Rarely malaria can spread by the inoculation of blood from an infected person to a healthy person. In this type of malaria, asexual forms are directly inoculated into the blood and pre-erythrocytic development of the parasite in the liver does not occur. Therefore, this type of malaria has a shorter incubation period and relapses due to persisting exoerythrocytic forms do not occur.

***2.5.2: Mother to the growing fetus (Congenital malaria):***

Transfer of parasitized red cells from infected mother to the child either transplacentally or during labor can lead to malaria in the newborn, called as congenital malaria.[32] Congenital malaria seems to be rarely reported and has always been considered to be more frequent in the nonimmune population than in the endemic areas. In recent years, however, higher prevalence of congenital malaria ranging from (8) % to (33) % has been reported from both malaria-

endemic and nonendemic areas, including the United States, Europe, India, etc.[32-33] Congenital malaria has been reported due to all four plasmodium species that commonly infect humans, although most cases are reported following *P. falciparum or P. vivax* malaria in the mother.[33,34] In nonendemic countries, *P. malariae* may cause a disproportionately higher number of congenital malaria cases due to its longer persistence in the host. Congenital malaria occurs more often during the first pregnancy. [33]

### *2.5.3: Transfusion Malaria:*

Malaria can be transmitted by transfusion of blood from infected donors. First reported in 1911, transfusion malaria is one of the most common transfusion-transmitted infections today. [35, 36] The risk of acquiring transfusion malaria is very low (1 case per 4 million) in nonendemic countries such as the United States, whereas in the endemic countries, it is much higher (>50 cases per million donor units). [36, 37]

Following a malaria infection, the individual may remain infective for weeks to months, or even years, in case of *P. malariae* infection. Therefore, those who have suffered from malaria should not donate blood for at least (3) years after becoming asymptomatic and proven carriers of *P. malariae* should never donate blood.

The risk of transmission is higher in transfusion of fresh, whole blood, particularly when the blood has been stored for less than (5) days and the risk is considerably lesser after (2) weeks.[38] The risk of transmission is extremely low in case of transfusions of plasma, plasma components, or derivatives devoid of intact red cells.[39,40]

It is difficult to identify malarial infection in donated blood specimens. Detection of such low parasitaemia is difficult or impossible with the peripheral smear examination or with more sensitive tests such as the antigen or polymerase chain reaction (*PCR*) assays. [41-42] The development of automated

protein microarray-based technology has the potential to further enhance antibody/antigen sensitivity. [38] There are reports of successful use of *RDTs* in screening donated blood. [43]

In non-endemic countries, donor deferral in combination with screening for specific antimalarial immunoglobulin provides an effective means of minimizing the risk of transmission. In endemic countries, more specific donor questioning, consideration of seasonal variation and geographical distribution may help to identify the population of donors who are most likely to be infected. In addition, the administration of antimalarials to transfusion recipients may help to prevent transmission. [42] It is also important to ensure that blood collected in highly endemic regions is not transfused to patients from areas of low endemicity.[39] In endemic countries, chemoprophylaxis was found to be particularly useful for protecting young children with no or little malarial immunity from developing acute and potentially fatal posttransfusion malaria.[41]

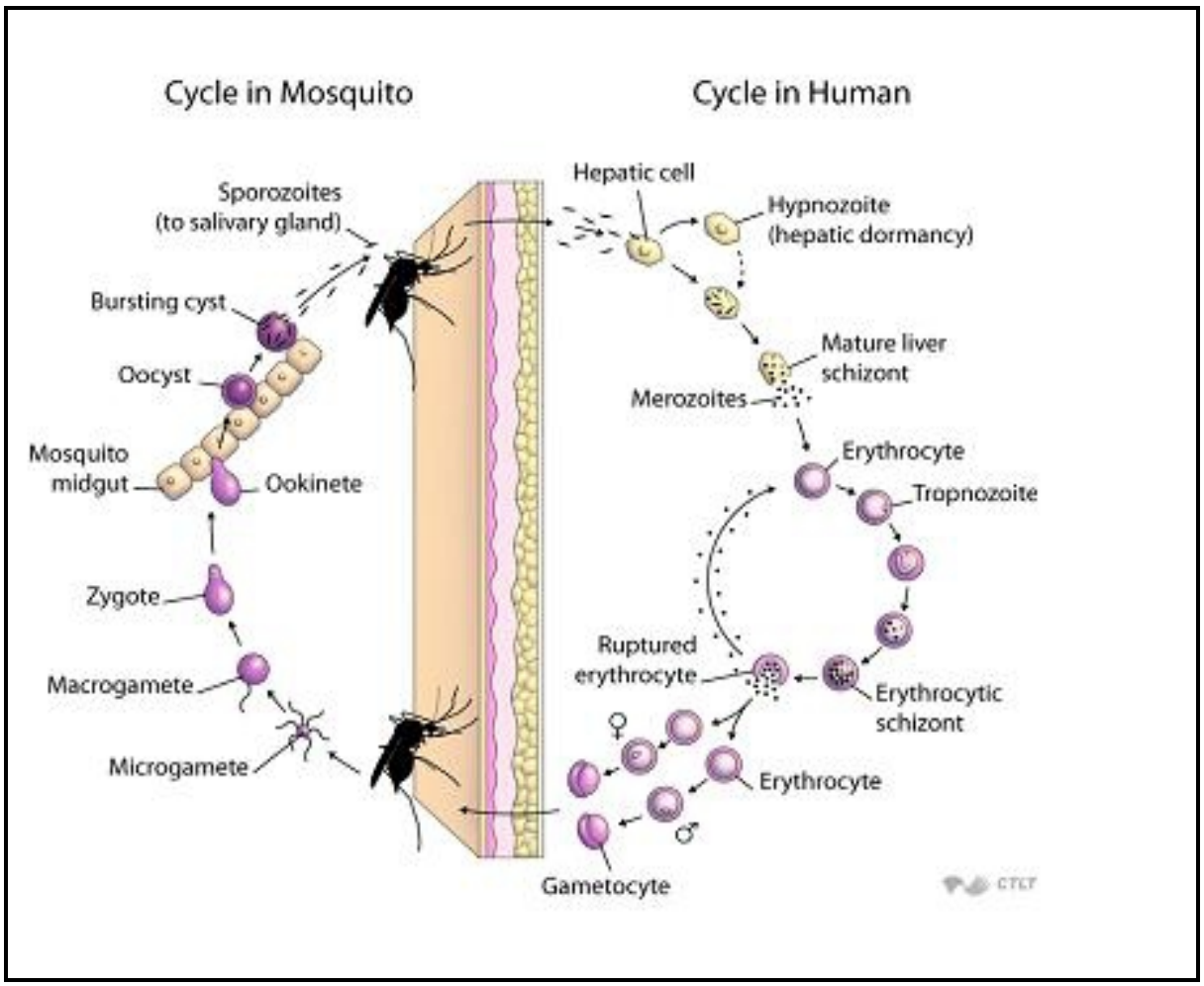
Transfusion malaria manifests with a shorter incubation period of (2–4) days as the inoculum contains the erythrocytic forms of the parasite and pre-erythrocytic phase of the life cycle within the liver does not occur. Typical symptoms of fever, malaise, and headache occur and in case of *P. falciparum* infection, particularly in the nonimmune patients, the infection can progress rapidly into fatal illness. Diagnosis of transfusion malaria requires a keen sense of clinical suspicion and any recipient of blood transfusion developing such symptoms should be tested immediately for malaria. As the transfusion-acquired *P. vivax* malaria does not have the exoerythrocytic phase, relapses do not occur. [44-45]

### *2.5.4: Needle sticks injury:*

Cases of malaria transmission through needle-stick injuries, accidentally among health care professionals (some even fatal) or due to needle sharing among drug addicts, have also been reported. [45]

## *2.6: Life Cycle:*

The malaria parasite has a complex, multistage life cycle occurring within two living beings, the vector mosquitoes and the vertebrate hosts. The survival and development of the parasite within the invertebrate and vertebrate hosts, in intracellular and extracellular environments, is made possible by a toolkit of more than (5,000) genes and their specialized proteins that help the parasite to invade and grow within multiple cell types and to evade host immune responses.[46,47] The parasite passes through several stages of development such as the sporozoites (Gr. Sporos = seeds; the infectious form injected by the mosquito), merozoites (Gr. Meros = piece; the stage invading the erythrocytes), trophozoites (Gr. Trophes = nourishment; the form multiplying in erythrocytes), and gamaetocytes (sexual stages) and all these stages have their own unique shapes and structures and protein complements. The surface proteins and metabolic pathways keep changing during these different stages that help the parasite to evade the immune clearance, while also creating problems for the development of drugs and vaccines. [47]




***(Fig.1):*** Life Cycle of plasmodium. (Source: http://ocw.jhsph.edu/)

Mosquitoes are the definitive hosts for the malaria parasites; where in the sexual phase of the parasite's life cycle occurs. The sexual phase is called *sporogony* and results in the development of innumerable infecting forms of the parasite within the mosquito that induce disease in the human host following their injection with the mosquito bite.

When the female Anopheles draws a blood meal from an individual infected with malaria, the male and female gamaetocytes of the parasite find their way into the gut of the mosquito. The molecular and cellular changes in the gamaetocytes help the parasite to quickly adjust to the insect host from the warm-blooded human host and then to initiate the sporogonic cycle. The male and female gametes fuse in the mosquito gut to form zygotes, which

subsequently develop into actively moving ookinetes that burrow into the mosquito midgut wall to develop into oocysts. Growth and division of each oocyst produces thousands of active haploid forms called sporozoites. After the sporogonic phase of (8–15) days, the oocyst bursts and releases sporozoites into the body cavity of the mosquito, from where they travel to and invade the mosquito salivary glands. When the mosquito thus loaded with sporozoites takes another blood meal, the sporozoites get injected from its salivary glands into the human bloodstream, causing malaria infection in the human host. It has been found that the infected mosquito and the parasite mutually benefit each other and thereby promote transmission of the infection. The *Plasmodium-infected mosquitoes* have a better survival and show an increased rate of blood-feeding, particularly from an infected host. [48, 49]

### *2.6.1: Schizogony in the Human Host:*

Man is the intermediate host for malaria, wherein the asexual phase of the life cycle occurs. The sporozoites inoculated by the infested mosquito initiate this phase of the cycle from the liver, and the latter part continues within the red blood cells, which results in the various clinical manifestations of the disease.

#### *Pre-erythrocytic Phase - Schizogony in the Liver:*

With the mosquito bite, tens to a few hundred invasive sporozoites are introduced into the skin. Following the intradermal deposition, some sporozoites are destroyed by the local macrophages, some enter the lymphatics, and some others find a blood vessel. [50–51] The sporozoites that enter a lymphatic vessel reach the draining lymph node wherein some of the sporozoites partially develop into exoerythrocytic stages [50] and may also prime the *T cells* to mount a protective immune response. [52]

The sporozoites that find a blood vessel reach the liver within a few hours. It has recently been shown that the sporozoites travel by a continuous

sequence of stick-and-slip motility, using the thrombospondin-related anonymous protein *(TRAP)* family and an actin–myosin motor.[53,54] The sporozoites then negotiate through the liver sinusoids, and migrate into a few hepatocytes, and then multiply and grow within parasitophorous vacuoles. Each sporozoite develop into a schizont containing (10,000–30,000) merozoites (or more in case of *P. falciparum*).[55–56] The growth and development of the parasite in the liver cells is facilitated by a favorable environment created by the circumsporozoite protein of the parasite.[57,58] The entire pre-erythrocytic phase lasts about (5–16) days depending on the parasite species: [59] on an average (5-6) days for *P. falciparum*, (8) days for *P. vivax*, (9) days for *P. ovale*, (13) days for *P. malariae* and (8-9) days for *P. knowlesi*.

The merozoites that develop within the hepatocyte are contained inside host cell-derived vesicles called merosomes that exits and keeps the liver intact, thereby protecting the merozoites from phagocytosis by Kupffer cells. These merozoites are eventually released into the blood stream at the lung capillaries and initiate the blood stage of infection thereon. [51]

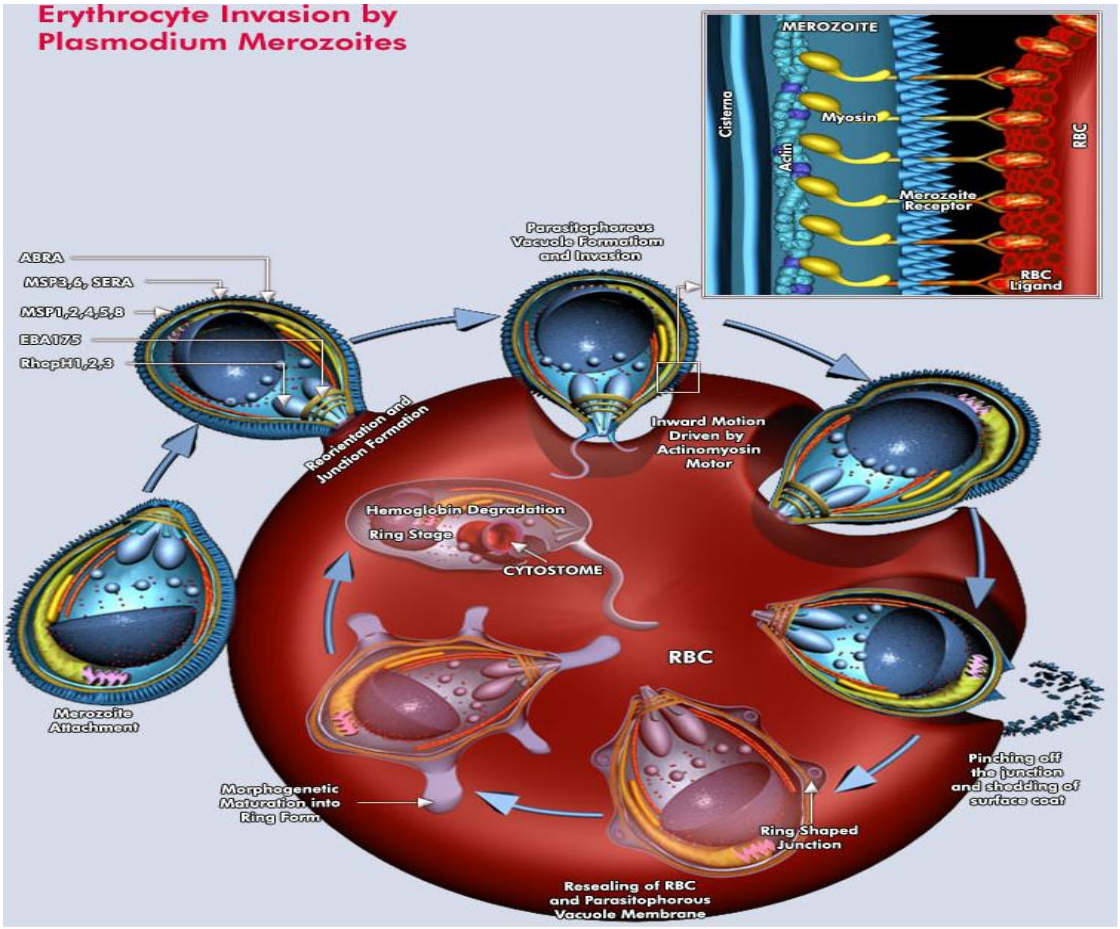
In *P. vivax and P. ovale* malaria, some of the sporozoites may remain dormant for months within the liver. Termed as hypnozoites, these forms develop into schizonts after some latent period, usually of a few weeks to months. It has been suggested that these late developing hypnozoites are genotypically different from the sporozoites that cause acute infection soon after the inoculation by a mosquito bite, [60, 61] and in many patients cause relapses of the clinical infection after weeks to months.

### *2.6.2: Erythrocytic Schizogony - Centre Stage in Red Cells:*

Red blood cells are the 'centre stage' for the asexual development of the malaria parasite. Within the red cells, repeated cycles of parasitic development occur with precise periodicity, and at the end of each cycle, hundreds of fresh daughter parasites are released that invade more number of red cells.

The merozoites released from the liver recognize, attach, and enter the red blood cells *(RBCs)* by multiple receptor–ligand interactions in as little as (60) seconds. This quick disappearance from the circulation into the red cells minimizes the exposure of the antigens on the surface of the parasite, thereby protecting these parasite forms from the host immune response. [62] The invasion of the merozoites into the red cells is facilitated by molecular interactions between distinct ligands on the merozoite and host receptors on the erythrocyte membrane. P. vivax invades only Duffy blood group-positive red cells, using the Duffy-binding protein and the reticulocyte homology protein, found mostly on the reticulocytes. The more virulent *P. falciparum* uses several different receptor families and alternate invasion pathways that are highly redundant. Varieties of Duffy binding-like *(DBL)* homologous proteins and the reticulocyte binding-likehomologous proteins of *P. falciparum* recognize different *RBC* receptors other than the Duffy blood group or the reticulocyte receptors. Such redundancy is helped by the fact that *P. falciparum* has four Duffy binding-like erythrocyte-binding protein genes, in comparison to only one gene in the *DBL-EBP* family as in the case of *P. vivax*, allowing *P. falciparum* to invade any red cell.[63,64]

The process of attachment, invasion, and establishment of the merozoite into the red cell is made possible by the specialized apical secretory organelles of the merozoite, called the micronemes, rhoptries, and dense granules. The initial interaction between the parasite and the red cell stimulates a rapid "wave" of deformation across the red cell membrane, leading to the formation of a stable parasite–host cell junction. Following this, the parasite pushes its way through the erythrocyte bilayer with the help of the actin–myosin motor, proteins of the thrombospondin-related anonymous protein family *(TRAP)* and aldolase, and creates a parasitoporous vacuole to seal itself from the host-cell cytoplasm, thus creating a hospitable environment for its development within the red cell. At this stage, the parasite appears as an intracellular "ring". [62, 65, 66]




***(Fig.2):*** Process of Invasion of Red Cells by Merozoites.

(Source: © 2009 GIAGEN).

Within the red cells, the parasite numbers expand rapidly with a sustained cycling of the parasite population. Even though the red cells provide some immunological advantage to the growing parasite, the lack of standard biosynthetic pathways and intracellular organelles in the red cells tend to create obstacles for the fast-growing intracellular parasites. These impediments are overcome by the growing ring stages by several mechanisms: by restriction of the nutrient to the abundant haemoglobin, by dramatic expansion of the surface area through the formation of a tubovesicular network, and by export of a range of remodeling and virulence factors into the red cell. [51] Haemoglobin from the red cell, the principal nutrient for the growing parasite, is ingested into

a food vacuole and degraded. The amino acids thus made available are utilized for protein biosynthesis and the remaining toxic heme is detoxified by heme polymerase and sequestrated as *haemozoin* (malaria pigment). The parasite depends on anaerobic glycolysis for energy, utilizing enzymes such as *pLDH,* plasmodium aldolase etc. As the parasite grows and multiplies within the red cell, the membrane permeability and cytosolic composition of the host cell is modified. [67,68] These new permeation pathways induced by the parasite in the host cell membrane help not only in the uptake of solutes from the extracellular medium but also in the disposal of metabolic wastes, and in the origin and maintenance of electrochemical ion gradients. At the same time, the premature haemolysis of the highly permeabilized infected red cell is prevented by the excessive ingestion, digestion, and detoxification of the host cell haemoglobin and its discharge out of the infected *RBCs* through the new permeation pathways, thereby preserving the osmotic stability of the infected red cells. [67, 68]

The erythrocytic cycle occurs every (24) hours in case of *P. knowlesi*, (48) hrs in cases of *P. falciparum, P. vivax* and *P. ovale* and *(72) hrs* in case of *P. malariae*. During each cycle, each merozoite grows and divides within the vacuole into (8–32) average of (10) fresh merozoites, through the stages of ring, trophozoite, and schizont. At the end of the cycle, the infected red cells rupture, releasing the new merozoites that in turn infect more *RBCs.* With sunbridled growth, the parasite numbers can rise rapidly to levels as high as (10) per host. [46]

A small proportion of asexual parasites do not undergo schizogony but differentiate into the sexual stage gamaetocytes. These male or female gamaetocytes are extracellular and nonpathogenic and help in transmission of the infection to others through the female anopheline mosquitoes, wherein they continue the sexual phase of the parasite's life cycle. Gamaetocytes of *P. vivax* develop soon after the release of merozoites from the liver, whereas in case of *P. falciparum,* the gamaetocytes develop much later with peak densities of the

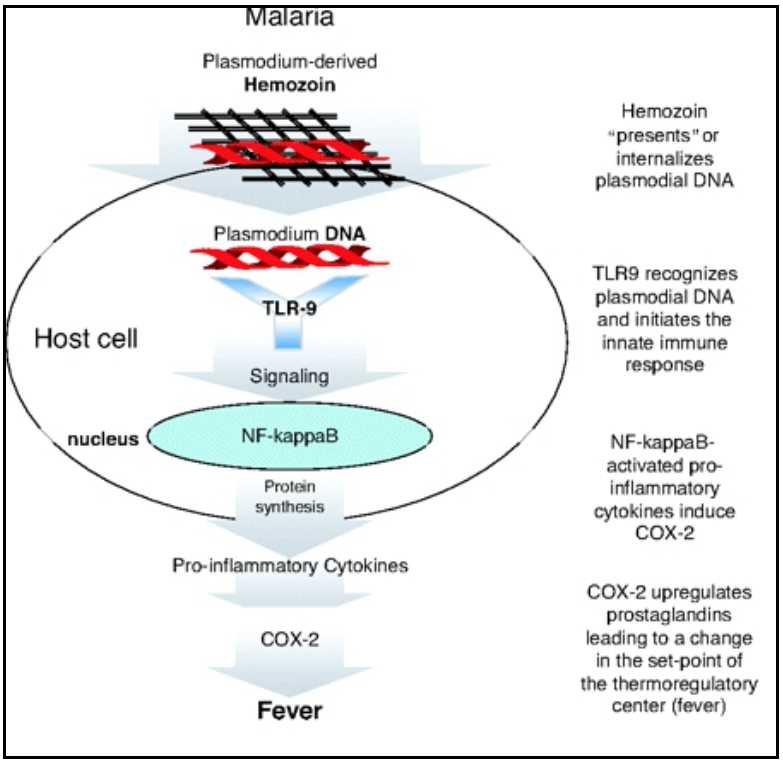
sexual stages typically occurring (1) week after peak asexual stage densities. [69, 70]

## *2.7: Pathogenesis of Malaria:*

The manifestations of malarial illness are caused by the infection of the red blood cells by the asexual forms of the malaria parasite and the involvement of the red cells makes malaria a potentially multisystem disease, as every organ of the body is reached by the blood. [71, 72] All types of malaria manifest with common symptoms such as fever, some patients may progress into severe malaria. Although severe malaria is more often seen in cases of *P. falciparum* infection, complications and even deaths have been reported in *non-falciparum malaria* as well.

At the completion of the schizogony within the red cells, each cycle lasting (24-72) hours depending on the species of the infecting parasite, newly developed merozoites are released by the lysis of infected erythrocytes and along with them, numerous known and unknown waste substances, such as red cell membrane products, haemozoin pigment, and other toxic factors such as glycosylphosphatidylinositol *(GPI)* are also released into the blood. These products, particularly the *GPI,* activate macrophages and endothelial cells to secrete cytokines and inflammatory mediators such as tumour necrosis factor, interferon-γ, interleukin-1, IL-6, IL-8, macrophage colony-stimulating factor, and lymphotoxin, as well as superoxide and nitric oxide (NO). Many studies have implicated the *GPI* tail, common to several merozoite surface proteins such as *MSP-1, MSP-2, and MSP-4*, as a key parasite toxin.[73,74] The systemic manifestations of malaria such as headache, fever and rigors, nausea and vomiting, diarrhoea, anorexia, tiredness, aching joints and muscles, thrombocytopaenia, immunosuppression, coagulopathy, and central nervous system manifestations have been largely attributed to the various cytokines released in response to these parasite and red cell membrane products.[75] In

addition to these factors, the plasmodial *DNA* is also highly proinflammatory and can induce cytokinaemia and fever. The plasmodial *DNA* is presented by haemozoin (produced during the parasite development within the red cell) to interact intracellularly with the Toll-like receptor-9, leading to the release of proinflammatory cytokines that in turn induce *COX-2*-upregulating prostaglandins leading to the induction of fever. [76, 77] Haemozoin has also been linked to the induction of apoptosis in developing erythroid cells in the bone marrow, thereby causing anaemia. [78, 79]



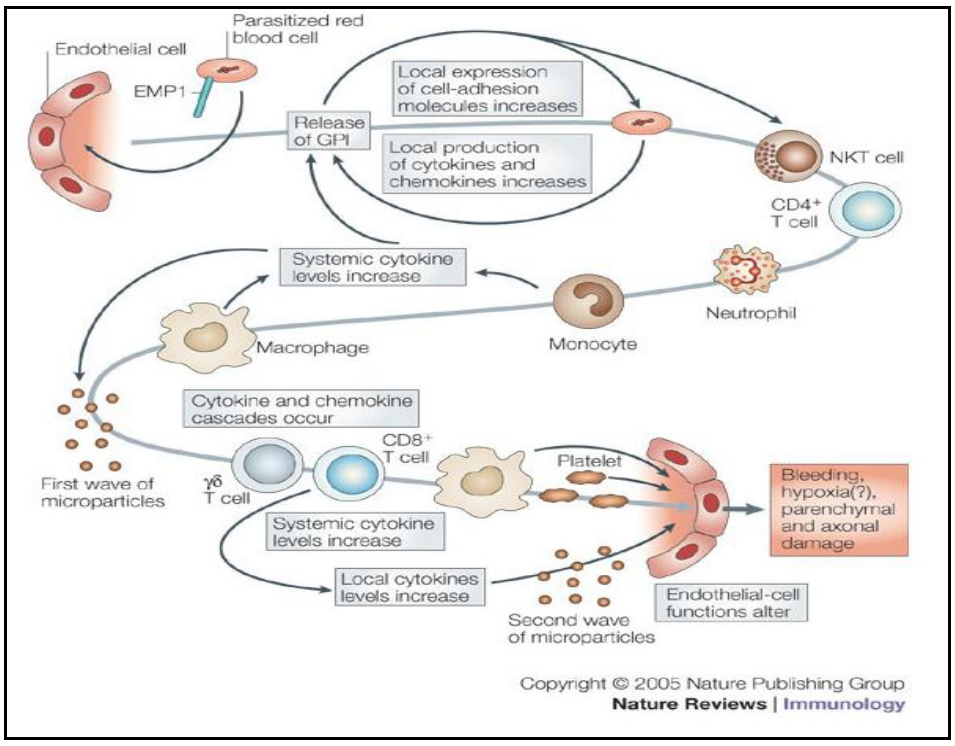

***(Fig.3):*** Induction of fever by malaria parasites.

(Source: Ralf R. Schumann. Malaria fever Hemzoin is involved but Toll free. PNAS 6 February 2007 ;104(6):1743-1744)

### *2.7.1: Pathogenesis of Severe Malaria*

The infection of the red cells by malaria parasites, particularly *P. falciparum*, results in progressive and dramatic structural, biochemical, and mechanical modifications of the red cells that can worsen into life-threatening complications of malaria. While the vast majority of severe malaria and related mortality are caused by *P. falciparum* infection, complications can occur in non-

falciparum infections as well. In recent years, several cases of severe infection and even deaths have been reported following infections with *P. vivax and P. knowlesi* infections.[80] Several pathophysiological factors such as the parasite biomass; 'malaria toxin (s)' and inflammatory response; cytoadherence, rosetting and sequestration; altered deformability and fragility of parasitized erythrocytes; endothelial activation, dysfunction and injury; and altered thrombostasis have been found to be involved in the development of severe malaria. All these phenomena are more profound and wide spread in *P. falciparum* infection compared to *non-falciparum infections*. As a result, except for severe anaemia, complications such as cerebral malaria, hypoglycaemia, metabolic acidosis, renal failure, and respiratory distress are more commonly seen in *P. falciparum* infections [81]




**(Fig.4):** Schematic representation of pathogenesis of severe malaria. (Source: Louis Schofield, Georges E. Grau. Immunological process in malaria pathogenesis. Nature Reviews Immunology September 2005;5.722- 735)

### *2.7.2: Parasite biomass:*

With its wide array of receptor families and highly redundant, alternate invasion pathways,[82] *P. falciparum* has the ability to invade *RBCs* of all ages, and with repeated cycles of development within the red cells, the parasite numbers exponentially grow into very high parasite burdens if the infection is uninhibited by treatment or host immunity. On the contrary, *P. vivax* preferentially infects only young *RBCs*, thus limiting its reproductive capacity and resultant parasite loads. Thus, the parasite load in *P. falciparum* infections can be very high, even exceeding (20-30) %, whereas in vivax malaria it rarely exceeds (2) % of total RBCs, even in case of severe disease.[81]

### *2.7.3: Role of cytokines in severe malaria:*

The cytokines of the proinflammatory cascade like tumor necrosis factor, interleukins, interferon-γ, and nitric oxide act as double-edged swords in the pathogenesis of malaria. Cytokines act as haemeostatic agents and an early proinflammatory cytokine response helps in limiting the infection, with the cytokines inhibiting the growth of malarial parasites in lower concentrations. On the other, failure to down-regulate this inflammatory response results in progressive immune pathology, leading to complications. Excessive levels of cytokines can lead to decreased mitochondrial oxygen use and enhanced lactate production; increased cytoadherence that in turn causes microvascular obstruction and more hypoxia; disturbed auto-regulation of local blood flow leading to poor circulation and further tissue hypoxia; dyserythropoiesis, poor red cell deformability and multifactorial anaemia; reduced gluconeogensis and hypoglycaemia; myocardial depression and cardiac insufficiency; loss of endothelial integrity and vascular damage in the lungs and brain; selective upregulation of vascular and intercellular adhesion molecules *(ICAMs),* particularly in the brain and placenta leading to cerebral malaria and placental

dysfunction; and activation of leukocytes and platelets, promoting procoagulant activity.[72,83] It can therefore be said that the outcome of malaria infection is determined by the balance between the pro- and anti-inflammatory cytokines.[72,84]

Some of the complications seen in *P. vivax* malaria may be related to cytokine-mediated injury. *P. vivax* has been reported to induce a greater inflammatory response than *P. falciparum* (with equal or greater parasite load), resulting in higher levels of cytokine release. The pyrogenic threshold is also lower in *P. vivax infections,* resulting in fever at lower levels of parasitaemia. Structural differences in the *P. vivax GPI* that may make it more pyrogenic and/or greater concentrations of Toll-like receptor-9-stimulating *motifs* within *P. vivax haemozoin* may be responsible for this greater pyrogenicity. A cholesterol/triglyceride (s)-containing lipid, that has greater activity than *GPI-like* phospholipids, has also been proposed as a *putative* malaria toxin unique to *P. vivax*, and that may also contribute to the pyrogenicity of *P. vivax*. [85].

***Cytoadherence, Sequestration, and Rosetting:***

Structural changes in the infected red cells and the resulting increase in their rigidity and adhesiveness are major contributors to the virulence for *P. falciparum* malaria. Owing to the increased adhesiveness, the red cells infected with late stages of *P. falciparum* (during the second half of the 48 hour life cycle) adhere to the capillary and postcapillary venular endothelium in the deep microvasculature (**cytoadherence**). The infected red cells also adhere to the uninfected red cells, resulting in the formation of red cell rosettes (**rosetting**). Cytoadherence leads to **sequestration** of the parasites in various organs such as the heart, lung, brain, liver, kidney, intestines, adipose tissue, subcutaneous tissues, and placenta. Sequestration of the growing *P. falciparum* parasites in these deeper tissues provides them the microaerophilic venous environment that is better suited for their maturation and the adhesion to endothelium allows

them to escape clearance by the spleen and to hide from the immune system. These factors help the *falciparum parasites* to undergo unbridled multiplication, thereby increasing the parasite load to very high numbers. Due to the sequestration of the growing parasites in the deeper vasculature, only the ring-stage trophozoites of *P. falciparum* are seen circulating in the peripheral blood, while the more mature trophozoites and schizonts are bound in the deep microvasculature, hence seldom seen on peripheral blood examination. If the cytoadherence-rosetting-sequestration of infected and uninfected erythrocytes in the vital organs goes on uninhibited, it ultimately blocks blood flow, limits the local oxygen supply, hampers mitochondrial *ATP synthesis*, and stimulates cytokine production - all these factors contributing to the development of severe disease [75,86,87]

Certain proteins expressed on the surface of the infected red cells mediate the adhesion of parasitized *RBCs* to the endothelium and to uninfected red cells. The most important of such proteins is the *P. falciparum* erythrocyte membrane protein (1) *(PfEMP1),* an antigenically diverse protein family that is expressed on the thousands of knob-like excrescences on the surface of red cells infected with *P. falciparum* trophozoites and schizonts. *PfEMP1* is anchored at the red cell membrane skeleton by the knob-associated histidine-rich protein. *PfEMP1* appears on the surface of the *P. falciparum*-infected red cells about 16 hours after the invasion and that heralds the cytoadherence.[88] *PfEMP1* can bind to several adhesion receptors expressed on the endothelial cells such as thrombospondin, *CD36, ICAM-1*, vascular cell adhesion molecule 1, platelet/EC adhesion molecule)/CD31, neural cell adhesion molecule, P-selectin and E-selectin, integrin αvβ3, globular C1q receptor (gC1qR)/hyaluronan binding protein 1/p32, chondroitin sulfate A *(CSA),* and hemagglutinin, and such binding can proceed synergistically. Whereas *ICAM-1* and *CD36* are more commonly used receptors, *CSA* acts as the receptor for binding in the placenta. Activation of endothelial cells by cytokines as well as by the parasitized *RBCs*

increases the expression of adhesion-promoting molecules and further promotes cytoadherence.[89] Differences in binding to these *receptors (CD36 and ICAM-1)* may determine the virulence of *P. falciparum* isolates from different parts of the world.[88]

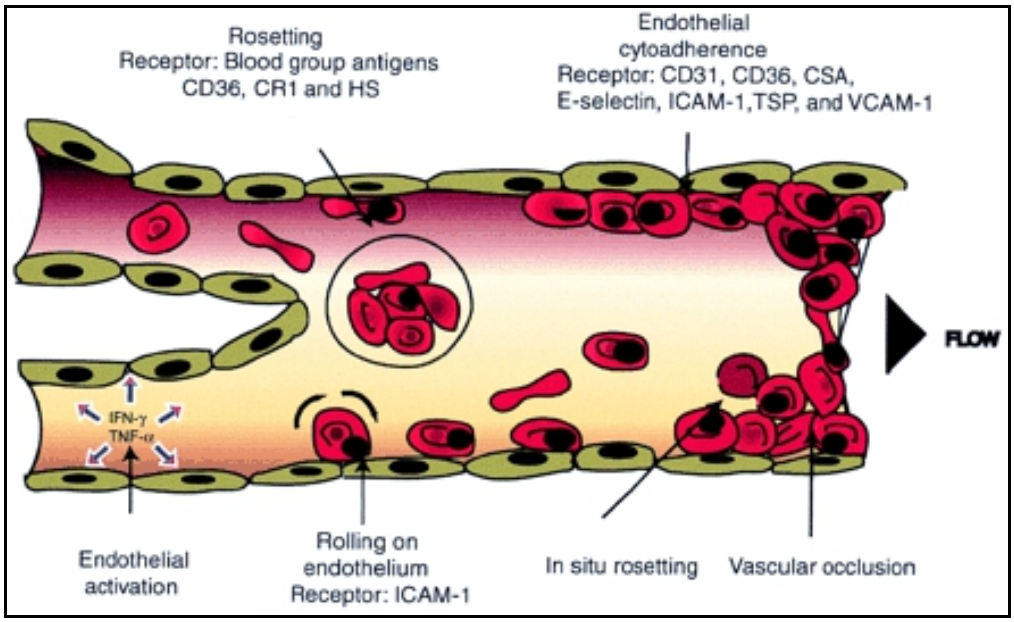
Rosetting is mediated by binding of *PfEMP1-DBLα* on the surface of infected red cells to complement receptor 1, CD31, and heparan sulfate-like glycosaminoglycans of uninfected *RBCs*.[90] Rosetting is found to be lesser in blood *group O* erythrocytes compared with *groups A, B, and AB*, and thus patients with blood *group O* may be protected from severe malaria.[91,92]

### *2.7.4: Red Cell Membrane Rigidity and Deformity:*

Altered red cell membrane rigidity and deformitability also contribute to the pathogenesis of severe malaria. In patients with severe *falciparum malaria*, the entire red cell mass, comprising mostly of unparasitized red cells and also parasitized red cells, becomes rigid.[93,94] Several mechanisms such as haemin-induced oxidative damage of the red cell membrane, alterations in the phospholipid bilayer and attached spectrin network by the proteins transported to the red cell membrane, thermally driven membrane fluctuations due to fever, and inhibition of the *Na+/K+* pump on the red cell membrane, possibly by nitric oxide *(NO)* may be responsible for the increase in rigidity and reduction in deformability of the red cells in falciparum malaria.[94,95] Reduced red cell deformability leads to increased splenic clearance and loss of red cells, causing anemia. Haemolysis, suppression of erythropoeisis by cytokines, and haemozoin-induced apoptosis in developing erythroid cells also contribute to the development of anemia in severe malaria.[94] Compared to infection with *P. falciparum*, in which red cell deformability is reduced, the red cell deformability is increased in *P. vivax* infection. While this may enable *P. vivax* infected red cells to survive the passage through the splenic sinusoids, the accompanying increase in fragility of both infected and noninfected red cells may contribute to

severe anemia in *P. vivax* malaria. Increased deformability of *P. vivax* infected red cells also makes sequestration and obstruction to blood flow unlikely. [96]

The pathogenesis of severe malaria therefore involves a cascading interaction between parasite and red cell membrane products, cytokines and endothelial receptors, leading to inflammation, activation of platelets, haemostasis, a procoagulant state, microcirculatory dysfunction and tissue hypoxia, resulting in various organ dysfunctions manifesting in severe malaria.[97]




**(Fig.5):** Cytoadherence and rosetting in post capillary vasculature. (Source: Qijun Chen, Martha Schlichtherle, Mats Wahlgren.Molecular Aspects of sever malaria Clinical microbiology review July 2000;13(3)439-450).

### *2.8: Immunity against Malaria:*

During its complex, multi-stage life cycle, the malaria parasite not only expresses a great variety of proteins at different stages, but these proteins also keep changing often. As a result, a natural infection with malaria parasites leads to only a partial and short lived immunity that is unable to protect the individual against a new infection. The complex interplay of parasite proteins with the immune system of the host has also made it difficult or even impossible to develop an effective vaccine against the disease until now. Immunity against

malaria can be classified into natural or innate immunity and acquired or adaptive immunity.

### *2.8.1: Natural or innate immunity to malaria:*

It is an inherent refractoriness of the host that prevents the establishment of the infection or an immediate inhibitory response against the introduction of the parasite. The innate immunity is naturally present in the host and is not dependent on any previous infection. Alterations in the structure of haemoglobin or in certain enzymes have been found to confer protection against either the infection or its severe manifestations and these traits are often found in areas of high malaria transmission. Duffy negativity in red cells protects against *P. vivax infection.* It is found to be widely prevalent in Africa and this may be responsible for the virtual elimination of this parasite from the continent. Certain thalassaemias (50% reduction in infection), homozygote *hemoglobin C* (90% reduction), haemoglobin E, and ovalocytosis carrier status have been reported to confer protection against *P. falciparum* or *P. vivax*. Glucose -6- phosphate dehydrogenase deficiency (50% protection) and sickle cell haemoglobin (90% protection) confer protection against severe malaria and related mortality. [98, 99]

Acute malarial infection also induces immediate, non-specific immune response that tends to limit the progression of disease. The humoral and cellular mechanisms of this 'nonspecific' defense are poorly defined. Primordial, extrathymic *T Cells* [Natural Killer *(NK)* $1.1^{-}$, intermediate *TCR (TCR$^{int}$)* cells] and autoantibody producing *B-1 cells* have been considered as the prime movers of this response. Natural killer *(NK)* cells are found in blood, in secondary lymphoid organs as well as in peripheral non-lymphoid tissues. Related cell types probably playing a role in innate malaria immunity are the *NKT* cells which in mice carry both the *NK1.1* surface marker and *T-cell* receptors *(TCR). NK cells* have been shown to increase in numbers and to be able to lyse

*P. falciparum-infected erythrocytes* in vitro. *NK cells* in peripheral blood produce Interferon-gamma in response to Plasmodium infected erythrocytes, leading to parasiticidal macrophage activation, and this may be of greater importance for innate malaria immunity than their potential to lyse infected host erythrocytes. These cells are also important in the initiation and development of adaptive immune responses. *NK cells* induce the production of the *proinflammatory chemokine Interleukin-8*, that in turn plays its role in the recruitment and the activation of other cells during malaria infection. Dendritic cells, macrophages, gamma delta *T cells* and *NKT* cells also sense the presence of the parasite and participate in the immune response. *NKT cells* are potent inhibitors of liver-stage parasite replication in mouse malaria systems in vitro. *NK1.1 CD4 murine T cells* have been reported to regulate *IgG a*ntibody responses to glycosylphosphatidyl inositol-anchored *P. falciparum* protein, and this may be important for a rapid, specific but major histocompatibility complex *(MHC)* unrestricted parasite control. Malaria infection gives rise to strongly elevated blood concentrations of non-malaria-specific immunoglobulin, but the importance of the underlying polyclonal *B-cell* activation for innate immunity is not known. [100]

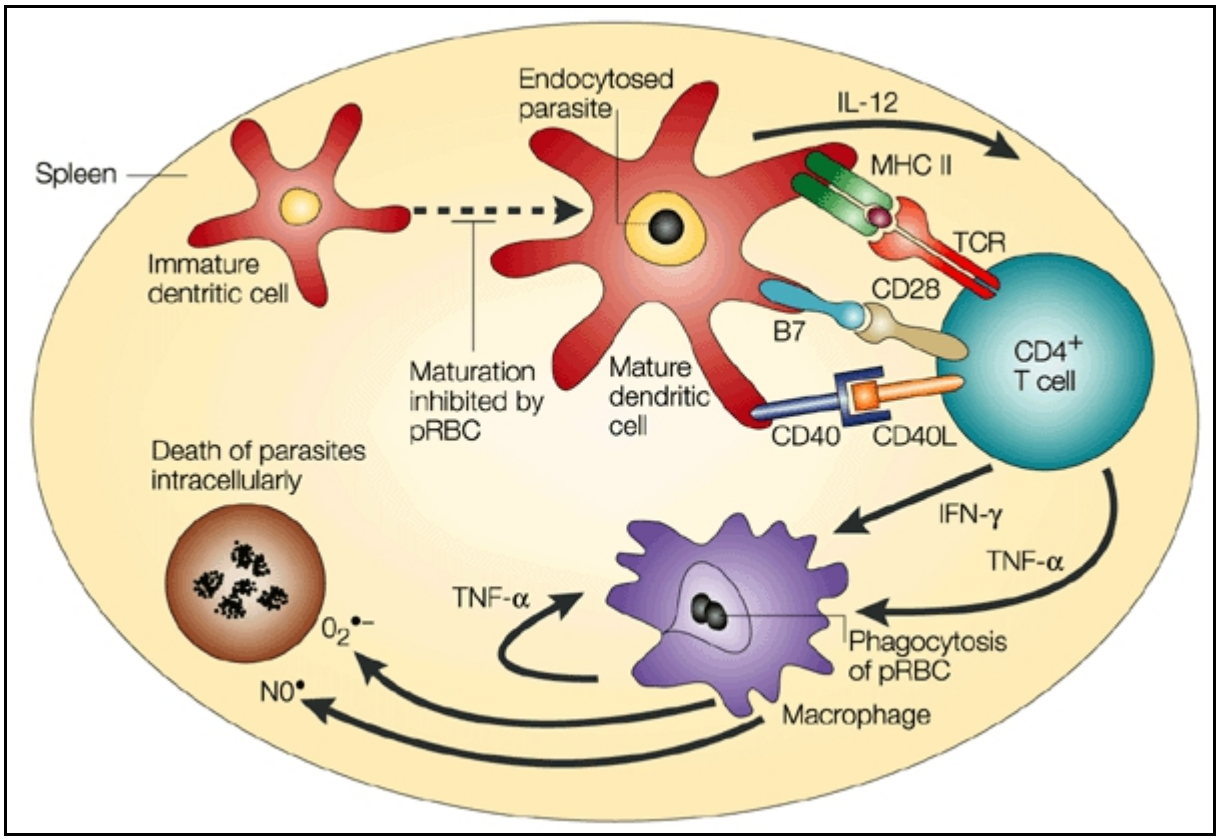
### *2.8.2: Acquired or adaptive immunity against malaria:*

Develops after infection and its protective efficacy varies depending on the characteristics of the host, place of stay, number of infections suffered etc. It has been graded as anti-disease immunity (that protects against clinical disease), anti-parasite immunity (protects against high parasitaemia), and sterilizing immunity (protects against new infections by maintaining a low-grade, asymptomatic parasitaemia; also called premunition), with a considerable overlap between these. Following infection with malaria parasites, a nonimmune individual commonly develops an acute clinical illness with very low levels of parasitaemia and the infection may progress to severe disease and

death. After a couple of more infections, anti-disease immunity develops and causes suppression of clinical symptoms even in the presence of heavy parasitaemia and also reduces the risk of severe disease. Frequent and multiple infections slowly lead to the development of anti-parasite immunity those results in very low or undetectable parasitaemia. Sterilizing immunity, though never fully achieved, results in a high degree of immune responsiveness, low levels of parasitaemia, and an asymptomatic carrier status. Premunition suggests an immunity mediated directly by the presence of the parasites themselves and not as much the result of previous infections. [98.99]

The presence of genetically and antigenically distinct strains of the parasites in a given locality and the occurrence of clonal antigenic variation during the course of an infection force the host to mount immune response against these different strains and antigenic variants. The acquisition of immunity against malaria is, therefore, very slow and not very effective and remains species specific and strain specific. However, in areas with stable endemic malaria and intense malaria transmission, such as Sub-Saharan Africa and forest areas in the Indian states like Orissa, Chhattisgarh, Jharkhand, southern Madhya Pradesh, and northeastern states etc., acquired immunity develops at a very early age. In these areas, children born to immune mothers are protected against disease during their first half year of life by maternal antibodies. This passive immunity is followed by (1 or 2) years of increased susceptibility before acquisition of active immunity. The risk of clinical disease increases from birth to about (6) months of age, depending on the transmission rate, and beginning at around (3 to 4) months of age, infants become susceptible to severe disease and death. The risk of cerebral malaria increases with age in children (2 to 4) years old. At about (2 to 5) years of age, due to repeated and frequent infections, the frequency of clinical disease begins to diminish and the risk of mortality sharply decreases, and by adulthood, most inhabitants generally possess sterilizing immunity. On the other, people living in unstable

endemic areas tend to acquire only partial immunity. [98, 99,101] Thus, the level of Antimalarial immunity influences the clinical outcome of the disease in different locations and age groups.




**(Fig.6):** Cellular immunity against malaria. (Source: Michael F. Good. Towards a blood – stage vaccine for malaria: are we following all the leads? Nature Reviews Immunology November 2011:1:117- 125).

The underlying mechanisms and antigenic specificity of protective immunity against malaria are not well understood. The acquired anti malaria immunity has been demonstrated to be strain specific and stage specific, with cross reactivity. Immune response has been documented against the various parasite antigens in pre-erythrocytic (sporozoite), asexual erythrocytic (merozoite) and sexual stages (gamaetocytes). Natural exposure to sporozoites does not induce complete (sterilizing) antiparasite and antidisease immunity but only limit the density of parasitaemia and thereby decrease the malaria-

associated morbidity and mortality. The acquired immunity is directed predominantly against the asexual erythrocytic stage, the primary targets being the extracellular merozoites in circulation. Although the pre-erythrocytic stage is also targeted by protective immune responses, it does not effectively block sporozoite invasion or intrahepatic development of the parasite. [99]

Malaria infection induces both polyclonal and specific immunoglobulin production, predominately *IgM and IgG* but also of other immunoglobulin isotypes. Of these, (5) % or more represent species- as well as stage-specific antibodies reacting with a wide variety of parasite antigens. Passive transfer of *IgG* from immune donors may be protective by reducing parasitaemia and clinical disease. Malaria infections of both humans and experimental animals are also associated with elevations in total *IgE* and *IgE anti-malarial antibodies*, reflecting a switch of regulatory *T cell* activities from *(Th1) to (Th2)* due to repeated exposure of the immune system to the parasites. *IgE* levels are significantly higher in patients with cerebral or other forms of severe disease than in those with uncomplicated malaria and the pathogenic effect of *IgE* is probably due to local overproduction in microvessels of tumour necrosis factor *(TNF)* and nitric oxide *(NO)* caused by *IgE-containing immune complexes*.[101]

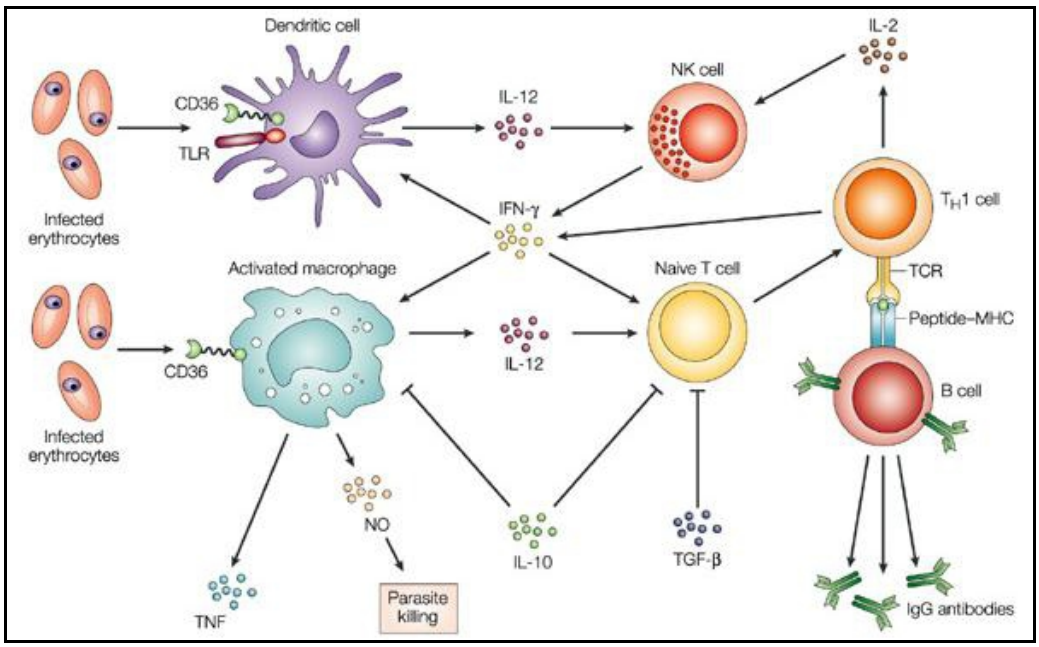
Antibodies may protect against malaria by a variety of mechanisms. They may inhibit merozoite invasion of erythrocytes and intra-erythrocytic growth or enhance clearance of infected erythrocytes from the circulation by binding to their surface, thereby preventing sequestration in small vessels and promoting elimination by the spleen. Opsonization of infected erythrocytes significantly increases their susceptibility to phagocytosis, cytotoxicity and parasite inhibition by various effector cells such as neutrophils and monocytes/macrophages. Interaction of opsonized erythrocytes with these effector cells induces release of factors such as *TNF* which may cause tissue lesions but which are also toxic for the parasites. [101]

Cell-mediated immune responses induced by malaria infection may protect against both pre-erythrocytic and erythrocytic parasite stages. *CD4 T cells* are essential for immune protection against asexual blood stages in both murine and human malaria. However the role of *CD8 T cells*, which have important effector functions in pre-erythrocytic immunity and which contribute to protection against severe malaria, is less clear. It has been proposed that *CD8 T cells* may regulate immunosuppression in acute malaria and down-modulate inflammatory responses. As human erythrocytes do not express *MHC* antigens, lysis of infected erythrocytes by *CD8 cytotoxic T lymphocytes* has no role in the defense against blood-stage parasites. [101].

Malaria parasites not only escape the host's immune response, owing to their antigenic diversity and clonal antigenic variation, but also modulate the immune response and cause significant immune suppression. The parasitized red cells, with the deposited haemozoin inside, have been found to inhibit the maturation of antigen presenting dendritic cells, thereby reducing their interaction with *T cells*, resulting in immunosuppression. Immune suppression in malaria increases the risk of secondary infections (such as nontyphoidal Salmonella, herpes zoster virus, hepatitis B virus, Maloney leukaemia virus and nematode infections and reactivation of Epstein-Barr virus) and may also reduce the immune response to certain vaccines. [102,103]

The acquired anti malaria immunity does not last long. In the absence of re-infection for about (6) months or (1) year, as may happen when the person leaves the malarious area, the acquired immunity turns ineffective and the individual becomes vulnerable to the full impact of a malarial infection once again. The immunity is also rendered less effective during pregnancy, particularly during the first and second pregnancies, due to the physiological immunosupression as well as the cytoadherence of erythrocytes to the newly available Chondroitin Sulfate A receptors on the placenta. Such loss of acquired immunity makes the pregnant woman more susceptible to malaria and its

complications. [98, 99] Immunosuppression in *HIV/AIDS* also increases the risks of clinical malaria, its complications and death. [104].




***(Fig.7):*** Regulation of adaptive immunity to blood-stage malaria.(Source: Mary M. Stevenson, Eleanor M. Riley. Innate immunity to malaria. Nature Reviews Immunology March 2004;4:169 – 180)

### *2.9: Clinical Features & Complications of Malaria:*

Malaria is a febrile illness characterized by fever and related symptoms. However it is very important to remember that malaria is not a simple disease of fever, chills and rigors. In fact, in a malarious area, it can present with such varied and dramatic manifestations that malaria may have to be considered as a differential diagnosis for almost all the clinical problems! Malaria is a great imitator and trickster, particularly in areas where it is endemic.

All the clinical features of malaria are caused by the erythrocytic schizogony in the blood. The growing parasite progressively consumes and degrades intracellular proteins, principally haemoglobin, resulting in formation of the 'malarial pigment' and haemolysis of the infected red cell. This also alters the transport properties of the red cell membrane, and the red cell becomes more spherical and less deformable. The rupture of red blood cells by merozoites

releases certain factors and toxins (such as red cell membrane lipid, glycosyl phosphatidyl inositol anchor of a parasite membrane protein), which could directly induce the release of *cytokines* such as *TNF a*nd *interleukin-1* from macrophages, resulting in chills and high grade fever. This occurs once in (48) hours, corresponding to the erythrocytic cycle. In the initial stages of the illness, this classical pattern may not be seen because there could be multiple groups (broods) of the parasite developing at different times, and as the disease progresses, these broods synchronize and the classical pattern of alternate day fever is established. It has been observed that in primary attack of malaria, the symptoms may appear with lesser degree of parasitaemia or even with submicroscopic parasitaemia. However, in subsequent attacks and relapses, a much higher degree of parasitaemia is needed for onset of symptoms. Further, there may be great individual variations with regard to the degree of parasitaemia required to induce the symptoms.

The first symptoms of malaria after the pre-patent period (period between inoculation and symptoms, the time when the sporozoites undergo schizogony in the liver) are called the ***primary attack.*** It is usually atypical and may resemble any febrile illness. As the disease gets established, the patient starts getting relapse of symptoms at regular intervals of (48-72) hours. The primary attack may spontaneously abort in some patients and the patient may suffer from relapses of the clinical illness periodically after (8-10) days owing to the persisting blood forms of the parasite. These are called as ***short term relapses (recrudescences).*** Some patients will get ***long term relapses*** after a gap of (20-60) days or more and these are due to the reactivation of the hypnozoites in the liver in case of *vivax* and *ovale malaria*. In *falciparum* and *malariae infections*, recrudescences can occur due to persistent infection in the blood.

### *2.9.1: Manifestations of acute malarial illness:*

While most of the clinical manifestations of malaria are caused by the malarial infection per se, high grade fever as well as the side effects of anti malarial therapy can also contribute to the clinical manifestations. All these may act in unison, further confusing the picture. In some cases, secondary infections like pneumonia or urinary tract infection can add to the woes. All these facts should always be kept in mind.

### *2.9.2: Typical features:*

The characteristic, text-book picture of malarial illness is not commonly seen. It includes three stages viz. cold stage, hot stage and Sweating stage. The febrile episode starts with shaking chills, usually at mid-day between (11) a.m. to (12) noon, and this lasts from (15) minutes to (1) hour (the cold stage), followed by high grade fever, even reaching above (106) $^{0}$ F, which lasts (2 to 6) hours (the hot stage). This is followed by profuse sweating and the fever gradually subsides over (2-4) hours. These typical features are seen after the infection gets established for about a week. The febrile paroxysms are usually accompanied by headaches, vomiting, delirium, anxiety and restlessness. These are as a rule transient and disappear with normalization of the temperature.

In *vivax malaria*, this typical pattern of fever recur once every (48) hours and this is called as Benign Tertian malaria. Similar pattern may be seen in ovale malaria too (Ovale tertian malaria). In falciparum infection (Malignant tertian malaria), this pattern may not be seen often and the paroxysms tend to be more frequent (Sub-tertian). In *P. malariae* infection, the relapses occurs once every (72) hours and it is called Quartan malaria.

### *2.9.3: Atypical features:*

Atypical features are more common in the following situations:

- *Falciparum malaria.*

- Early infection.
- Patients at extremes of age.
- Patients who are immune-compromised (extremes of age, malnourished, *AIDS*, tuberculosis, cancers, on immunosuppressive therapy etc.).
- Patients on chemoprophylaxis for malaria.
- Patients who have had recurrent attacks of malaria.
- Patients with end stage organ failure.
- Last but not the least, pregnancy.

### *2.9.4: Typical fever:*

In an endemic area, it is rather unusual to find cases with typical fever pattern. Some patients may not have fever at all and may present with other symptoms listed below. Many present with fever of various patterns - low grade to high grade, with or without chills, intermittent to continuous, or even as cases of prolonged fever. In the initial stages of the illness, fever may be quotidien, with more than one spike per day and this is due to the development of multiple broods of the parasite. As the disease progresses, these broods get synchronized and the fever tends to be more uniform. However in cases of *P. falciparum* malaria and mixed infections, this pattern of multiple spikes may continue.

### *2.9.5: Headache:*

Headache may be a presenting feature of malaria, with or without fever. It can be unilateral or bilateral. Sometimes the headache could be so intense that it may mimic intra-cranial infections or intra-cranial space occupying lesions. It may also mimic migraine, sinusitis etc. Presence of projectile vomiting, papilloedema, neck stiffness and focal neurological signs would suggest other possibilities.

### *2.9.6: Body ache, back ache and joint pains:*

These symptoms are fairly common in malaria. These can occur even during the prodromal period and at that stage these are generally ignored and diagnosis of malaria is impossible owing to lack of peripheral parasitaemia. They are also common accompaniments of the malaria paroxysm. Sometimes, malaria may present only with these symptoms, particularly in cases of recurrent malaria.

### *2.9.7: Dizziness, vertigo:*

Some patients may present with dizziness or vertigo, with or without fever. They may also have associated vomiting and/or diarrhoea. This may mimic labyrinthitis, Menniere's disease, vertebro-basilar insufficiency etc. Rarely patients may present with swaying and cerebellar signs. Drugs like chloroquine, quinine, mefloquine and halofantrine can also cause dizziness, vertigo, and tinnitus.

### *2.9.8: Altered behaviour:*

An acute psychosis: Patients may present with altered behaviour, mood changes, hallucinosis or even acute psychosis, with or without fever. Malaria may be detected accidentally in such cases and they improve completely with anti malarial therapy. Altered behaviour may also be due to high grade fever or drugs. Antimalarial drugs like chloroquine, quinine, mefloquine and halofantrine can cause restlessness, hallucinations, confusion, delirium or even frank psychosis.

### *2.9.9: Altered sensorium:*

Patients with *P. falciparum* malaria may present with altered sensorium due to severe infection, hypoglycaemia, electrolyte imbalance due to vomiting or diarrhoea (particularly the elderly), hyperpyrexia, subclinical convulsions etc. Differential diagnosis will include acute encephalitis, meningitis, metabolic

encephalopathy etc. As a rule of the thumb, malaria should be considered a possibility in all cases of acute neuropsychiatric syndromes and in cases of proven malaria, other possibilities should be considered in the presence of papilloedema, increases *ICT*, neck stiffness and focal deficits.

### *2.9.10: Convulsions, coma:*

Patients with cerebral malaria present with generalized seizures and deep unarousable coma. Sometimes one single fit can precipitate deep, coma. These could also be due to hypoglycaemia and all patients presenting with these manifestations should be administered (25-50) % dextrose immediately. Drugs like chloroquine, quinine, mefloquine and halofantrine may also trigger convulsions.

### *2.9.11: Cough:*

Cough may be a presenting feature of malaria, particularly *P. falciparum* infection. Patient may have pharyngeal congestion and features of mild bronchitis. Patients who have persistent cough and/or fever even after clearance of parasitaemia should be evaluated for secondary bacterial pneumonias/ bronchopneumonia and bronchitis.

### *2.9.12: Breathlessness:*

In severe *falciparum malaria*, patients may present with history of breathlessness, due to either severe anemia or non-cardiogenic pulmonary oedema. Secondary respiratory tract infections and lactic acidosis are other rarer causes for tachypnoea and/or breathlessness in these patients. Patients with pre-existing cardio-vascular or pulmonary compromise may deteriorate or even die if they suffer from severe malaria.

### *2.9.13: Chest pain:*

Acute retrosternal or precordial pain may be presenting feature of malaria. It may radiate to the left or right shoulder tips or arms. It is due to rapid

increase in the splenic size and perisplenitis. This pain may mimic acute myocardial infarction, pleurisy, neuralgia etc. Coupled with breathlessness, sweating and hypotension (algid malaria), the picture will very closely resemble that of acute (myocardial infarction) MI.

### *2.9.14: Acute abdomen:*

Patients can present with acute abdominal pain, guarding and rigidity, mimicking bowel perforation, acute appendicitis, acute cholecystitis, ureteric colic etc.

One such patient presented with pain abdomen and vomiting with low grade fever, and on examination had tenderness in the right lower abdomen. He was posted for appendicectomy. Preoperative blood test revealed *P. falciparum* malaria and he recovered completely with Antimalarial.

### *2.9.15: Weakness:*

Sometimes patients may present with history of weakness, malaise and prostration. On examination they may have significant pallor, hypotension, dehydration etc. Algid malaria may present like this and the patient may not have fever at all. Chloroquine is also known to cause profound muscular weakness and a new disease called macrophagic myofaciitis has been described in patients receiving chloroquine.

### *2.9.16: Vomiting and diarrhoea:*

Malaria can present as a case of acute gastroenteritis with profuse vomiting and watery diarrhoea (Choleraic form). Vomiting is very common in malaria and is due to high grade fever, the disease itself or even drugs. Vomiting may pose problems in administering antimalarial treatment. These could also be due to drugs like chloroquine and due to secondary bacterial or amoebic colitis.

### *2.9.17: Jaundice:*

Patients may present with history of yellowish discoloration of eyes and urine. Mild jaundice is fairly common in malaria and may be seen in (20-40) % of the cases. Deeper jaundice with serum bilirubin of more than (3) mg/dL is seen in severe *P. falciparum* malaria and is associated with anaemia, hyperparasitaemia and malarial hepatitis with elevated serum enzymes. Malaria must be considered as a differential diagnosis for all cases of jaundice in a malarious area. [105]

### *2.9.18: Pallor:*

Severe anaemia can be a presenting feature of malaria. It is usually normocytic normochromic. It may pose special problems in pregnancy and in children. Pre-existing nutritional anaemia may be aggravated by malaria.

### *2.9.19: Puffiness of lids:*

Occasionally patients may present with puffiness of lids, with or without renal dysfunction.

### *2.9.20: Secondary infections:*

Malaria produces significant immune suppression and this can result in secondary infections. Common among them are pneumonia, aspiration bronchopneumonia (in the elderly), urinary tract infection, colitis etc. Meningitis and enteric fever have also been reported. In *falciparum malaria*, severe infection can lead to septicaemic shock (algid malaria). Persistence of fever, neutrophilic leucocytosis and focal signs of infection should always alert the clinician to this possibility of secondary infections.

### *2.9.21: Hepatosplenomegaly:*

Patients can present with enlargement of liver and/or spleen, tender or non-tender, with or without fever. Rapid enlargement of spleen or liver in malaria can cause acute pain in the abdomen or chest. Generally, organomegaly

is noticed in the second week of malarial illness. However, in cases of relapse or recrudescence, it may be present earlier. Also, in immune compromised patients splenomegaly may be absent. In pregnancy, particularly second half, splenomegaly may be smaller or an enlarged spleen may regress in size due to immune suppression. Although splenomegaly is a cardinal sign of malaria, absence of splenomegaly does not rule out the possibility of malaria.[105]

### *2.9.22: Combinations of the above:*

Patients can frequently present with various combinations of the above mentioned symptoms and signs, further confusing the picture. This list is not exhaustive and malaria may present in many other ways. In all the above listed situations, patients may not have associated fever, thus confusing the picture. In some, fever may follow these symptoms. Therefore, one should not wait for the typical symptoms of malaria to get a blood test done; it is always better to do a smear whenever reasonable doubt exists. [106]

### *2.9.23: Complications:*

There are several serious complications of malaria. Among these is the development of respiratory distress, which occurs in up to (25) % of adults and (40) % of children with severe *P. falciparum malaria*. Possible causes include respiratory compensation of metabolic acidosis, noncardiogenic pulmonary oedema, concomitant pneumonia, and severe anaemia. Although rare in young children with severe malaria, acute respiratory distress syndrome occurs in (5–25) % of adults and up to (29) % of pregnant women. Co infection of *HIV* with malaria increases mortality. Renal failure is a feature of black water fever, where haemoglobin from lysed red blood cells leaks into the urine. Infection with *P. falciparum* may result in cerebral malaria, a form of severe malaria that involves encephalopathy. It is associated with retinal whitening, which may be a useful clinical sign in distinguishing malaria from other causes of fever. Splenomegaly, severe headache, hepatomegaly (enlarged liver), hypoglycaemia,

and haemoglobinuria with renal failure may occur. Malaria in pregnant women is an important cause of stillbirths, infant mortality and low birth weight, particularly in *P. falciparum infection*, but also with *P. vivax* [106].

### *2.10: The Liver*

#### *2.10.1: Liver Anatomy:*

The liver is a large and complex organ weighing approximately (1.2–1.5) kg in the healthy adult. It is located beneath and is attached to the diaphragm, is protected by the lower rib cage, and is held in place by ligamentous attachments. Despite the functional complexity of the liver, it is relatively simple in structure. It is divided unequally into two lobes by the falciform ligament, with the right lobe being approximately six times larger than the left lobe. There is no known functional difference between the lobes, and communication flows freely between all areas of the liver. [107]

Unlike most organs, which have a single blood supply, the liver is an extremely vascular organ that receives its blood supply from two sources: the hepatic artery and the portal vein. The hepatic artery, a branch of the aorta, supplies oxygen-rich blood from the heart to the liver and is responsible for providing approximately (25) % of the total blood supply to the liver. The portal vein supplies nutrient-rich blood (collected as food is digested) from the digestive tract, and it is responsible for providing approximately (75) % of the total blood supply to the liver. The two blood supplies eventually merge and flow into the sinusoids, which course between individual hepatocytes. Approximately (1,500) ml of blood passes through the liver per minute. The liver is drained by a collecting system of veins that empties into the hepatic veins and ultimately into the inferior vena cava. The excretory system of the liver begins at the bile canaliculi. The bile canaliculi are small spaces between the hepatocytes that form intrahepatic ducts, where excretory products of the cell can drain. The intrahepatic ducts join to form the right and left hepatic

ducts, which drain the secretions from the liver. The right and left hepatic ducts merge to form the common hepatic duct, which is eventually joined with the cystic duct of the gallbladder to form the common bile duct. Combined digestive secretions are then expelled into the duodenum. [107]

### *2.10.2: Liver Physiology:*

The various functions of the liver are carried out by the liver cells or hepatocytes. Currently, there is no artificial organ or device capable of emulating all the functions of the liver. Some functions can be emulated by liver dialysis, an experimental treatment for liver failure. The liver is thought to be responsible for up to (500) separate functions, usually in combination with other systems and organs. [108]

***Synthesis:***

A large part of amino acid synthesis.

**The liver performs several roles in carbohydrate metabolism:**

- Gluconeogenesis (the synthesis of glucose from certain amino acids, lactate or glycerol).
- Glycogenolysis (the breakdown of glycogen into glucose).
- Glycogenesis (the formation of glycogen from glucose) (muscle tissues can also do this). The liver is responsible for the mainstay of protein metabolism, synthesis as well as degradation.

**The liver also performs several roles in lipid metabolism:**

- Cholesterol synthesis.
- Lipogenesis, the production of triglycerides (fats).
- A bulk of the lipoproteins is synthesized in the liver.
- The liver produces coagulation factors I (fibrinogen), II (Prothrombin), V, VII, IX, X and XI, as well as protein C, protein S and antithrombin.

- In the first trimester fetus, the liver is the main site of red blood cell production. By the (32) nd week of gestation, the bone marrow has almost completely taken over that task.
- The liver produces and excretes bile (a yellowish liquid) required for emulsifying fats. Some of the bile drains directly into the duodenum, and some is stored in the gallbladder.
- The liver also produces *insulin-like growth factor 1 (IGF-1)*, a polypeptide protein hormone that plays an important role in childhood growth and continues to have anabolic effects in adults.
- The liver is a major site of thrombopoietin production. Thrombopoietin is a glycoprotein hormone that regulates the production of platelets by the bone marrow.

## *Breakdown:*

- The breakdown of insulin and other hormones.
- The liver glucouronidates bilirubin, facilitating its excretion into bile.
- The liver breaks down or modifies toxic substances (e.g., methylation) and most medicinal products in a process called drug metabolism. This sometimes results intoxication, when the metabolite is more toxic than its precursor. Preferably, the toxins are conjugated to avail excretion in bile or urine.
- The liver converts ammonia to urea (urea cycle) [(108)]

## *Other functions:*

- The liver stores a multitude of substances, including glucose (in the form of glycogen), vitamin A (1–2 years' supply), vitamin D (1–4 months' supply), vitamin $B_{12}$ (1-3 years' supply), iron, and copper.

- The liver is responsible for immunological effects- the reticuloendothelial system of the liver contains many immunologically active cells, acting as a 'sieve' for antigens carried to it via the portal system.
- The liver produces albumin, the major osmolar component of blood serum.
- The liver synthesizes angiotensinogen, a hormone that is responsible for raising the blood pressure when activated by renin, an enzyme that is released when the kidney senses low blood pressure.

**Among the most important liver functions are:**

- Removing and excreting body wastes and hormones as well as drugs and other foreign substances, these substances have entered the blood supply either through production by metabolism within the body or from the outside in the form of drugs or other foreign compounds. Enzymes in the liver alter some toxins so they can be more easily excreted in urine.
- Synthesizing plasma proteins, including those necessary for blood clotting Most of the (12) clotting factors are plasma proteins produced by the liver. If the liver is damaged or diseased, it can take longer for the body to form clots. Other plasma proteins produced by the liver include albumin which binds many water-insoluble substances and contributes to osmotic pressure, fibrinogen which is a key to the clotting process, and certain globulins which transport substances such as cholesterol and iron.
- Producing immune factors and removing bacteria, helping the body fight infection the phagocytes in the liver produce acute-phase proteins in response to microbes. These proteins are associated with the inflammation process, tissue repair, and immune cell activities. [108]

***Other important but less immediate functions include:***

Producing bile to aid in digestion Bile salts aid in fat digestion and absorption. Bile is continuously secreted by the liver and stored in the gallbladder until a meal, when bile enters the beginning of the small intestine.

Bile production ranges from (250) ml to (1) L per day depending of amount of food eaten.

**Excretion of bilirubin:** is one of the few waste products excreted in bile. Macrophages in the liver remove worn out red blood cells from the blood. Bilirubin then results from the breakdown of the haemoglobin in the red blood cells and is excreted into bile by hepatocytes. Jaundice results when bilirubin cannot be removed from the blood quickly enough due to gallstones, liver disease, or the excessive breakdown of red blood cells. [108]

Storing certain vitamins, minerals, and sugars the liver stores enough glucose in the form of glycogen to provide about a day's worth of energy. The liver also stores fats, iron, copper, and many vitamins including vitamins A, D, K, and $B_{12}$.

**Processing nutrients absorbed from digestive tract:** The liver converts glucose into glycogen, its storage form. This glycogen can then be transformed back into glucose if the body needs energy. The fatty acids produced by the digestion of lipids are used to synthesize cholesterol and other substances. The liver also has the ability to convert certain amino acids into others.

Despite the wide variety of functions performed by the liver, there is very little specialization among hepatocytes (liver cells). Aside from the macrophages called Kupffer cells in the liver, hepatocytes all seem to be able to perform the same wide variety of tasks.

One of the liver's most interesting abilities is self-repair and the regeneration of damaged tissues. In clearing the body of toxins, the liver is damaged by exposure to harmful substances, demonstrating why this capability is important. It also gives hope that if a failing liver can be supported for a

certain period of time, it might regenerate and allows the patient to survive and regain a normal life. (108)

### *2.10.3: Liver function tests:*

Liver function tests (*LFTs or LFs*), are groups of clinical biochemistry laboratory blood assays designed to give information about the state of a patient's liver,[109] The parameters measured include PT/INR, aPTT, albumin, bilirubin (direct and indirect) and others. According to some, liver transaminases *(AST/ALT (SGOT/SGPT)* are NOT liver function tests but are biomarkers of liver injury in a patient with some degree of intact liver function.

Other sources include transaminases.[110, 111] Most liver diseases cause only mild symptoms initially, but it is vital that these diseases be detected early. Hepatic (liver) involvement in some diseases can be of crucial importance. This testing is performed by a medical technologist on a patient's serum or plasma sample obtained by phlebotomy. Some tests are associated with functionality (e.g., albumin); some with cellular integrity (e.g., transaminase) and some with conditions linked to the biliary tract (gamma-glutamyl transferase and alkaline phosphatase). Several biochemical tests are useful in the evaluation and management of patients with hepatic dysfunction. These tests can be used to (1) detect the presence of liver disease, (2) distinguish among different types of liver disorders, (3) gauge the extent of known liver damage, and (4) follow the response to treatment. Some or all of these measurements are also carried out (usually about twice a year for routine cases) on those individuals taking certain medications- anticonvulsants are a notable example - in order to ensure that the medications are not damaging the person's liver. (111)

#### *2.10.3.1: Total Plasma Protein:*

Proteins and amino acids have unique structures that allow them to participate in some specific types of chemical reactions. Proteins are polymers consisting of amino acid units. Amino acid units within the protein are joined by

peptide bonds, giving the primary structure of proteins. The amino acid unit on the carboxyl end of the protein contains a free carboxyl group that does not participate in peptide bond formation. The amino acid unit on the amino end of the protein contains a free amino group that does not participate in peptide bond formation. These characteristics of proteins, the amino and carboxyl ends, and peptide bonding play a role in the methods of analysis for total serum proteins. Proteins are ampholytes, and in aqueous solutions they may have positive and negative charges on the same molecule. This property is used to separate protein molecules during electrophoresis. The *pH* of the solution determines the net charge of the molecule. At different *pH* environments, hydrogen ions will be gained or lost from the carboxyl and amine ends and from functional groups amino acids residues. Since proteins are composed of different amino acids, different proteins will gain or lose hydrogen ions at different *pH* environments.

In addition to their properties as ampholytes, proteins also have other representative structural properties based on their polymer makeup and bonding. Fibrous proteins are string like in configuration and usually function as structural components of the body, such as fibrinogen and collagen. Most plasma proteins and enzymes are globular proteins, which are spherical in configuration.

Serum contains a large variety of proteins and a large amount of total protein, averaging (7.0) g/dI in the adult. In contrast, protein levels in serum and urine are normally in the microgram or milligram per deciliter range. Methods for measuring proteins in body fluids are based upon the unique structural properties as well as their relative concentration in the body fluids. There are two main types of proteins, albumin and globulins. They are grouped into (5) classes as determined by their electrophoretic separation: albumin, alpha1 globulins, α2- globulins, beta globulins, and gamma globulins. Serum albumin, at around (4) g/dI, makes up roughly half of the total serum proteins. A simple way to assess the balance between the patient's albumin and globulins in serum

is to calculate the albumin: globulin, or *A/G, ratio*. Globulins (G) are calculated as albumin (A) subtracted from total serum proteins. Albumin is then divided by globulin. [112]

***Interpretation of Total Serum Protein Levels:***

Total serum protein levels are affected by not only changes in one or more of the individual protein levels, but also by changes in plasma water. A variety of conditions cause hyperproteinaemia, or increased serum protein. **Haemoconcentration**, or decreased plasma water volume, will cause total serum protein levels to be increased. Dehydration is the usual cause of haemoconcentration, which is secondary to a variety of conditions including diarrhea, severe vomiting, and water deprivation.

Increased total serum protein levels can also occur when there is an increase in a variety of immunoglobulin's following inflammation or infection or a monoclonal increase in immunoglobulins, such as in multiple myeloma.

Increased total protein can also result from measuring an unexpected protein such as fibrinogen. Serum is derived from clotted whole blood in which fibrinogen is removed in the clotting process. However, if incomplete clotting occurs before centrifugation, some fibrinogen can remain behind in the serum specimen. Hypoproteinaemia, or decreased protein levels in the blood, is often due to hypoalbuminaemia, since albumin is the most abundant single protein. Typical causes of hypoproteinaemia are starvation or nutritional deficiency of essential amino acids, renal loss such as in nephritic syndrome, gastrointestinal loss such as in enteropathy, or hepatic failure in which the liver is unable to synthesize proteins. [2] Serum protein electrophoresis results can also indicate inflammatory states of the liver in which there are elevated gamma globulin protein fractions, especially immunoglobulin A *(IgA)* and *IgM* levels. In cirrhosis, protein electrophoresis results show that fast-moving gamma

globulins often migrate in the beta to gamma region, causing a beta-gamma bridge. (112)

### *2.10.3.2: Albumin:*

Albumin is a protein made specifically by the liver, and can be measured cheaply and easily. It is the main constituent of total protein; the remaining fraction is called globulin (including the immunoglobulins). Albumin levels are decreased in chronic liver disease, such as cirrhosis. It is also decreased in nephrotic syndrome, where it is lost through the urine. Poor nutrition or states of impaired protein catabolism, such as in Ménétrier's disease, may also lead to hypoalbuminaemia. The half-life of albumin is approximately (20) days. Albumin is not considered to be an especially useful marker of liver synthetic function; coagulation factors are much more sensitive. (112)

### *Interpretation of Serum Albumin Levels:*

Albumin levels are affected not only by changes in albumin level, but also by changes in plasma water volume, in a manner similar to effects on total serum protein. In addition to dehydration, fluid redistribution such as in **ascites** may cause hypoalbuminaemia as well. (2) Hypoalbuminaemia from deficient protein level is due to loss, such as from the gastrointestinal system in malabsorption or protein-losing enteropathy, from the renal system as in glomerulonephritis or nephritic syndrome, or from skin due to severe burns. Hypoalbuminaemia can also result from increased catabolism as a result of tissue damage and inflammation, as found in neoplasms or autoimmunity. Decreased serum albumin is also associated with malnutrition and inadequate amino acid intake. Finally, hypoalbuminaemia is correlated with declining synthesis in the liver, as associated with cirrhosis or other situations of liver failure. (113)

***Other Serum Proteins***

There are many other serum proteins to consider. For example, transferrin is involved in the transport of iron in plasma and C-reactive protein (*CRP*) is an acute-phase reactant or inflammatory protein. (113)

***2.10.3.3: Serum Bilirubin:***

***Bilirubin Metabolism:***

Bilirubin is a degradation product of the heme portion of haemoglobin. Heme is degraded in cells of the reticuloendothelial system, mainly the spleen. The protoporphyrin ring of the heme is opened to the biliverdin form and iron is released. Biliverdin is reduced to produce the yellow-pigmented molecule bilirubin. The bilirubin molecule, a tetrapyrrole, has low solubility in water or plasma. When it is released into blood, it is bound to albumin for transport. Covalently bound bilirubin is called delta bilirubin. When the bilirubin-albumin form reaches the liver, it loses albumin and enters the hepatocyte. Within the hepatocyte, the liver enzyme uridyl diphosphate glucuronyltransferase (UDPG-transferase) transfers molecules of glucuronate, a sugar, to the bilirubin molecule. About (85) % of bilirubin is conjugated with two molecules of glucuronate to form diglucuronate-bilirubin. Most of the rest of bilirubin is conjugated with one sugar molecule to form monoglucuronate bilirubin. The addition of the sugar group increases the solubility of the molecule. Conjugated bilirubin passes into the intestine through the bile duct, where intestinal bacteria reduce bilirubin to urobilinogen. Some urobilinogen may be reabsorbed through the intestinal mucosa and returned to the portal circulation and the liver. The remaining urobilinogen is excreted into urine or oxidized to form urobilin and excreted in the feces. Urobilin is one component in feces giving its characteristic brown colour. (113)

***Total bilirubin (TBIL):***

Bilirubin is a breakdown product of heme (a part of hemoglobin in red blood cells). The liver is responsible for clearing the blood of bilirubin. It does this by the following mechanism: Bilirubin is taken up into hepatocytes, conjugated (modified to make it water-soluble), and secreted into the bile, which is excreted into the intestine.

Increased total bilirubin causes jaundice, and can signal a number of problems:

1. **Prehepatic**: Increased bilirubin production. This can be due to a number of causes, including hemolytic anaemias and internal haemorrhage.
2. **Hepatic**: Problems with the liver, which are reflected as deficiencies in bilirubin metabolism (e.g., reduced hepatocyte uptake, impaired conjugation of bilirubin, and reduced hepatocyte secretion of bilirubin). Some examples would be cirrhosis and viral hepatitis.
3. **Posthepatic**: Obstruction of the bile ducts, reflected as deficiencies in bilirubin excretion. (Obstruction can be located either within the liver or in the bile duct).

***Direct bilirubin (Conjugated Bilirubin):***

The diagnosis is narrowed down further by looking at the levels of direct bilirubin.

If direct (i.e. conjugated) bilirubin is normal, then the problem is an excess of unconjugated bilirubin, and the location of the problem is upstream of bilirubin excretion. Haemolysis, viral hepatitis, or cirrhosis can be suspected.

If direct bilirubin is elevated, then the liver is conjugating bilirubin normally, but is not able to excrete it. Bile duct obstruction by gallstones or cancer should be suspected. [(113)]

***Hyperbilirubinaemia:***

Bilirubin levels in the blood are increased as the result of several disorders or conditions. These disorders or conditions are categorized into three phases of bilirubin metabolism, prehepatic, hepatic, and posthepatic. Prehepatic hyperbilirubinaemia is caused by increased haemolysis and increased degradation of heme. Prehepatic hyperbilirubinaemia occurs in patients with sickle cell anaemia and other hemolytic diseases that cause increased destruction of red blood cells and release of haemoglobin. The typical serum bilirubin pattern of prehepatic hyperbilirubinaemia is increased unconjugated bilirubin and normal conjugated bilirubin.

Hepatic hyperbilirubinaemia is generally due to defective transport to the liver or conjugation of bilirubin in the hepatocytes. Disorders of transport into the hepatocytes or conjugation disorders result in increased unconjugated bilirubin. Examples of these conditions include Gilbert's and *Crigler-Najjar syndromes. Gilbert's syndrome* is a hereditary disorder in which there is decreased bilirubin transport into the hepatocytes. Several hepatic hyperbilirubinaemic disorders are caused by failure of the liver to conjugate bilirubin. *Crigler-Najjar syndrome* results from a hereditary deficiency of the *UDPG-transferase enzyme*. Neonatal jaundice is caused by the inability of the immature liver of the newborn to produce *UDPG-transferase*. A slight increase in bilirubin in the second and third days of life is a normal response. Damage to hepatocytes by hepatitis, cirrhosis, toxic substances, and other disorders can inhibit conjugation as well. The typical serum bilirubin pattern of hepatic hyperbilirubinaemia is increased unconjugated and conjugated bilirubin. Serum enzymes that indicate hepatocellular inflammation and cellular damage within the liver, including *ALT and AST,* are also often elevated.

Posthepatic hyperbilirubinaemia is generally due to a defect in transporting conjugated bilirubin and bile out of the liver. It can involve obstruction of the small canaliculi within the liver, the hepatic bile duct, and the

common bile duct leading to the duodenum of the small intestine. Posthepatic hyperbilirubinaemia is often called obstructive jaundice. Obstruction of the bile flow can be due to gallstones or to scarring and nodules, such as from cirrhosis or tumors. The typical serum bilirubin pattern of posthepatic hyperbilirubinaemia is increased conjugated bilirubin but normal unconjugated bilirubin. Serum enzymes that indicate biliary cell damage, including alkaline phosphatase and *GGT,* are also often elevated. [113].

***Obstructive Jaundice:***

Obstruction of bile from the liver may be caused by gallstones in the bile duct or a tumor that impedes the flow of bile into the intestine. Increased total bilirubin in the blood of a patient with obstructive jaundice is usually a reflection of increased conjugated bilirubin. Other effects of obstructive jaundice may be defective excretion of lipid substances through bile.

Obstructive jaundice is associated with increased levels of alkaline phosphatase and *GGT* and increased serum total and direct bilirubin. When the direct bilirubin level is nearing the total bilirubin level, this generally indicates a posthepatic hyperbilirubinaemia such as in obstructive jaundice. Sometimes other liver enzymes are elevated as well, indicating hepatic inflammation. Ultrasound and other imaging techniques are needed to locate the source of the obstruction.

***Neonatal hyperbilirubinaemia:***

Neonatal hyperbilirubinaemia may result from a variety of conditions, some of which are transient, such as neonatal physiological jaundice of the newborn, and some of which are more serious conditions that cause sustained hyperbilirubinaemia in the newborn. Prolonged hyperbilirubinaemia often indicates a serious condition in the neonate, such as haemolytic disease of the newborn, biliary atresia, or, in rare situations, idiopathic neonatal hepatitis. Biliary atresia is a rare congenital anatomic obstruction of the biliary ducts and

presents as posthepatic jaundice. Idiopathic neonatal hepatitis is a hepatic inflammatory condition of unknown cause and presents as hepatic jaundice, with elevated hepatic enzymes. It is also quite rare. In the patient, haemolytic disease of the newborn presents as a prehepatic jaundice with a positive direct antiglobulin (Coombs) test. It is most commonly attributed to blood group incompatibility between the expressed blood groups of the mother and the fetus. *Rh blood groups* are often involved. The disease process develops when the blood cells of the mother come in contact with incompatible cells of the fetus through transfusion or through contact with the infant's blood during pregnancy. The mother's immune system recognizes the incompatible cells as foreign and develops antibodies against them. If the immune process is begun when an infant is delivered, the antibodies will not affect that infant. However, antibody may be directed against blood cells of a subsequent pregnancy if that fetus also expresses an incompatible blood group. Haemolytic disease of the newborn may require exchange transfusion in the neonate if haemolysis is severe.

Phototherapy is often the treatment of choice when bilirubin levels exceed (10) mg/dL since kernicterus may occur at levels approaching (20) mg/dL. Phototherapy is a method of treating neonatal hyperbilirubinaemia in which the baby is placed periodically under a light source emitting (450)-nm wavelength light. Light diffuses through layers of skin and converts unconjugated bilirubin to stable water soluble forms that can be excreted. The baby's eyes are protected during this process from harmful ultraviolet *(UV)* and near-*UV rays*. Kernicterus is a condition in which brain cell nuclei stain yellow and become damaged due to bilirubin or other molecules. High levels of bilirubin are less likely to cause brain damage in adults due to the natural barrier in the brain, called the blood-brain barrier. Kernicterus typically occurs at bilirubin levels greater than (20) mg/dL in infants due to their immature blood-brain barrier. It may result in cerebral palsy, deafness, or mental retardation.

***Physiological Jaundice of the Newborn:***

It is typically due to the short-term or transient immaturity of the liver. This causes a short-term delay in ability to produce *UDPG-transferase* for conjugation. In addition, there is higher turnover of neonatal erythrocytes shortly after birth in order to replace fetal haemoglobin (*Hb F)* with haemoglobin (A). This causes an increase in supply of heme for degradation to bilirubin. The reference ranges listed in Test Methodology (7–3) show the slight increase in bilirubin in the first few days of life when compared to the normal adult bilirubin level. This peaks at around (2 to 4) days but may remain elevated for upto (2) weeks.

Hyperbilirubinaemia may be more pronounced due to blood group differences of mother and child, particularly with group O mothers, who make naturally occurring immunoglobulin M *(IgM)* anti-A and anti-B antibodies but may also make small amounts of immunoglobulin G (*IgG*) anti-A and anti-B antibodies, which can enter the maternal-fetal circulation. This generally produces only mild increase in fetal cell turnover but moderate hyperbilirubinaemia. Other factors that may influence neonatal hyperbilirubinaemia include decreased binding of unconjugated bilirubin to albumin, reabsorption of intestinal meconium, and constituents in mother's milk. Progesterone and other hormones in breast milk as well as beta glucuronidase may suppress neonatal conjugation of bilirubin. This factor slows excretion of water-soluble forms, thus promoting jaundice. [113]

***2.10.3.4: Alanine transaminase (ALT):***

Alanine transaminase (*ALT)*, also called Serum Glutamic Pyruvate Transaminase (*SGPT*) or Alanine aminotransferase (*ALAT)* is an enzyme present in hepatocytes (liver cells). When a cell is damaged, it leaks this enzyme into the blood, where it is measured. *ALT* rises dramatically in acute liver

damage, such as viral hepatitis or paracetamol (acetaminophen) overdose. Elevations are often measured in multiples of the upper limit of normal [114].

### *2.10.3.5: Aspartate transaminase (AST):*

Aspartate transaminase *(AST*) also called Serum Glutamic Oxaloacetic Transaminase *(SGOT*) or aspartate aminotransferase (*ASAT*) is similar to *ALT* in that it is another enzyme associated with liver parenchymal cells. It is raised in acute liver damage, but is also present in red blood cells and cardiac and skeletal muscle and is therefore not specific to the liver. The ratio of *AST* to *ALT* is sometimes useful in differentiating between causes of liver damage. [114,115] Elevated *AST* levels are not specific for liver damage, and *AST* has also been used as a cardiac marker.

### *2.10.3.6: Alkaline phosphatase (ALP):*

Alkaline phosphatase (*ALP*) is an enzyme in the cells lining the biliary ducts of the liver. *ALP* levels in plasma will rise with large bile duct obstruction, intrahepatic cholestasis or infiltrative diseases of the liver. *ALP* is also present in bone and placental tissue, so it is higher in growing children (as their bones are being remodeled) and elderly patients with Paget's disease. [116]

### *2.10.3.7: Gamma glutamyl transpeptidase (GGT):*

Although reasonably specific to the liver and a more sensitive marker for cholestatic damage than *ALP*, Gamma glutamyl transpeptidase *(GGT)* may be elevated with even minor, sub-clinical levels of liver dysfunction. It can also be helpful in identifying the cause of an isolated elevation in *ALP. (GGT* is raised in chronic alcohol toxicity). [116]

### *2.10.3.8: 5' Nucleotidase (5'NTD):*

5' Nucleotidase is another test specific for cholestasis or damage to the intra or extrahepatic biliary system, and in some laboratories, is used as a

substitute for *GGT* for ascertaining whether an elevated *ALP* is of biliary or extra-biliary origin. [116]

### *2.10.3.9: Coagulation test (e.g., INR):*

The liver is responsible for the production of coagulation factors. The *international normalized ratio (INR)* measures the speed of a particular pathway of coagulation, comparing it to normal. Increased levels of *INR* means that blood is taking more time than usual to coagulate or clot. The *INR* will be increased only if the liver is so damaged that synthesis of vitamin K-dependent coagulation factors has been impaired; it is not a sensitive measure of liver function. It is mainly used to monitor patients receiving warfarin anticoagulation. [117]

### *2.10.4: Pathophysiology of Liver Enzyme:*

Enzyme analysis is used to aid in diagnosis and treatment of disease. In particular, enzymes that are synthesized within cellular organelles carry out their functions within cells and are released into body fluids when those cells become diseased.

Thus, an increase in enzyme activity when compared to the reference range can indicate pathological changes in certain types of cells and tissues. Enzyme activity levels in body fluids can reflect leakage from cells due to cellular injury, or changes in enzyme production rate or actual enzyme induction due to metabolic or genetic states or proliferation of neoplasms. In the latter case, increased enzyme activity can be used as a tumor marker. One aspect of enzyme activity that must be considered is the relative time frame that enzyme activity appears in the blood plasma and how long it remains in relationship to the disorder. For example, some enzymes found in plasma due to tissue necrosis or inflammation rise at such a slow rate that they are not useful for early detection or treatment of the disease.

Other enzymes rapidly decline in circulation because of inactivation or metabolism.

The clinical utility of enzyme activity in relationship to specific tissue pathology and clinical signs is enhanced when the enzyme activity quickly rises following the onset of the disorder and remains elevated for an adequate time frame, particularly when other clinical signs and symptoms are not sufficient to provide a diagnosis. [113]

Damage to tissue can release different types of enzymes based on their location. For example, mild inflammation of the liver reversibly increases the permeability of the cell membrane and releases cytoplasmic enzymes such as lactate dehydrogenase *(LD),* alkaline phosphatase (*ALP), ALT, and AST*, while cellular death (necrosis) will release mitochondrial sources of *ALT* and *AST*.9 Distribution of these enzymes within specific types of hepatic tissues varies. *ALP and GGT* are more concentrated in the biliary ducts or tissues of the small ducts (canaliculi), while *AST, ALT, and LD* are found mainly in structural (parenchymal) hepatic cells. Multiple forms of enzymes, called isoenzymes, are distributed in several different tissue types. For example, *ALP* is found in hepatobiliary tissues but also found in all cytoplasmic membranes of all cells of the body, especially in osteoblasts (bone forming cells), intestinal mucosa, placenta, and renal tubules. Table (7–3) summarizes tissue origins of key liver enzymes. Methods of analysis for transaminases are presented in Test Methodology (7–4), and for (*GGT)* in Test Methodology (7–5). Correlation of patterns of hyperbilirubinaemia with serum enzymes may also be helpful. For example, prehepatic jaundice, as indicated by relatively normal serum conjugated bilirubin, increased unconjugated bilirubin, and increased urinary urobilinogen, correlates with normal serum levels of hepatocellular and hepatobiliary enzymes, with the exception of *LDH* and possibly *AST*. These two enzymes are found in erythrocytes so, in situations of increased red cell breakdown, these enzyme concentrations are elevated in the serum. Hepatic

jaundice, indicated by increased serum conjugated and unconjugated bilirubin and increased urinary urobilinogen, correlates with increased serum levels of hepatocellular enzymes. Posthepatic jaundice, as indicated by relatively normal serum unconjugated bilirubin, increased conjugated bilirubin, and decreased urinary urobilinogen, correlates with pronounced elevations of hepatobiliary enzymes but normal to slightly elevated serum levels of hepatocellular enzymes.

***Analytical Aspects of liver Enzymes:***

Multiple forms of enzymes exist, particularly when an enzyme is composed of two or more polypeptide chains or subunits. This unique composition of protein isomers, or protomers, can be associated with different distribution within tissues, and unique chemical and physical properties. Multiple forms of enzymes can also be produced as a result of postgenetic modification, such as from metabolism. The term isoenzyme refers to forms of the same enzyme that arise from unique gene sequences. Characterization of isoenzymes based on separation techniques can be used to indicate the tissue source of the enzyme activity and correlate with specific diseases. Separation and quantification techniques for isoenzymes and multiple forms of enzymes include zone electrophoresis, ion-exchange chromatography, selective inactivation, and immunoassay methods.

*ALP* can consist of multiple forms that may arise from a variety of tissues, but rarely are more than two forms found in a particular patient's sample. The enzyme appears to be associated with lipid transport in the intestine and calcium transport in bone. *ALP* is present at or near the cell membrane. Generally this enzyme is derived from two sources: bone osteoblasts and either liver, intestine, or placenta. Rarely, serum *ALP* is derived from renal tubules. Table (7–4) lists the common *ALP* isoenzymes and methods of their separation. Monospecific antisera can be used to specifically measure *ALP* isoenzymes, including placental and intestinal forms. [113]

### *2.11. The Kidney:*

#### *2.11.1: Anatomy:*

In humans the kidneys are located in the abdominal cavity, more specifically in the paravertebral gutter and lie in a retroperitoneal position at a slightly oblique angle. There are two, one on each side of the spine. The asymmetry within the abdominal cavity caused by the liver typically results in the right kidney being slightly lower than the left, and left kidney being located slightly more medial than the right.[118,119] The left kidney is approximately at the vertebral level *T12 to L3*,[120] and the right slightly lower. The right kidney sits just below the diaphragm and posterior to the liver, the left below the diaphragm and posterior to the spleen. Resting on top of each kidney is an adrenal gland. The upper (cranial) parts of the kidneys are partially protected by the $11^{th}$ and $12^{th}$ ribs, and each whole kidney and adrenal gland are surrounded by two layers of fat (the perirenal and pararenal fat) and the renal fascia. Each adult kidney weighs between (125 and 170) grams in males and between (115 and 155) grams in females, [121] and the left kidney is typically slightly larger than the right.[122]

The kidney has a bean-shaped structure; each kidney has a convex and concave surface. The concave surface, the renal hilum, is the point at which the renal artery enters the organ, and the renal vein and ureter leave. The kidney is surrounded by tough fibrous tissue, the renal capsule, which is itself surrounded by perinephric fat, renal fascia (of Gerota) and paranephric fat. The anterior (front) border of these tissues is the peritoneum, while the posterior (rear) border is the transversalis fascia.

The superior border of the right kidney is adjacent to the liver; and the spleen, for the left kidney. Therefore, both move down on inhalation.

The kidney is approximately (11–14) cm in length, (6) cm wide and (4) cm thick.

The substance, or parenchyma, of the kidney is divided into two major structures: superficial is the renal cortex and deep is the renal medulla. Grossly, these structures take the shape of (8 to 18) cone-shaped renal lobes, each containing renal cortex surrounding a portion of medulla called a renal pyramid (of Malpighi), [121] between the renal pyramids are projections of cortex called renal columns (of Bertin). Nephrons, the urine-producing functional structures of the kidney, span the cortex and medulla. The initial filtering portion of a nephron is the renal corpuscle, located in the cortex, which is followed by a renal tubule that passes from the cortex deep into the medullary pyramids. Part of the renal cortex, a medullary ray is a collection of renal tubules that drain into a single collecting duct.

The tip, or papilla, of each pyramid empties urine into a minor calyx; minor calyces empty into major calyces, and major calyces empty into the renal pelvis, which becomes the ureter.

***Blood supply:***

The kidneys receive blood from the renal arteries, left and right, which branch directly from the abdominal aorta. Despite their relatively small size, the kidneys receive approximately (20) % of the cardiac output,[121] each renal artery branches into segmental arteries, dividing further into interlobar arteries which penetrate the renal capsule and extend through the renal columns between the renal pyramids. The interlobar arteries then supply blood to the arcuate arteries that run through the boundary of the cortex and the medulla. Each arcuate artery supplies several interlobular arteries that feed into the afferent arterioles that supply the glomeruli.

The **interstitum** (or **interstitium**) is the functional space in the kidney beneath the individual filters (glomeruli) which are rich in blood vessels. The interstitum absorbs fluid recovered from urine. Various conditions can lead to scarring and congestion of this area, which can cause kidney dysfunction and

failure. After filtration occurs the blood moves through a small network of venules that converge into interlobular veins. As with the arteriole distribution the veins follow the same pattern, the interlobular provide blood to the arcuate veins then back to the interlobar veins which come to form the renal vein exiting the kidney for transfusion for blood.

***Innervation:***

The kidney and nervous system communicate via the renal plexus, whose fibers course along the renal arteries to reach the kidney.[123] Input from the sympathetic nervous system triggers vasoconstriction in the kidney, thereby reducing renal blood flow. The kidney is not thought to receive input from the parasympathetic nervous system. Sensory input from the kidney travels to the (10-11) levels of the spinal cord and is sensed in the corresponding dermatome. Thus, pain in the flank region may be referred from the kidney.[123]

***2.11.2: Renal Physiology:***

Renal physiology (Latin Renes, "kidney") is the study of the physiology of the kidney. This encompasses all functions of the kidney, including reabsorption of glucose, amino acids, and other small molecules; regulation of sodium, potassium, and other electrolytes; regulation of fluid balance and blood pressure; maintenance of acid-base balance; the production of various hormones including erythropoietin, and the activation of vitamin D

Much of renal physiology is studied at the level of the nephron, the smallest functional unit of the kidney. Each nephron begins with a filtration component that filters blood entering the kidney. This filtrate then flow along the length of the nephron, which is a tubular structure lined by a single layer of specialized cells and surrounded by capillaries. The major functions of these lining cells are the reabsorption of water and small molecules from the filtrate into the blood, and the secretion of wastes from the blood into the urine.

Proper function of the kidney requires that it receives and adequately filters blood. This is performed at the microscopic level by many hundreds of thousands of filtration units called renal corpuscles, each of which is composed of a glomerulus and a Bowman's capsule. A global assessment of renal function is often ascertained by estimating the rate of filtration, called the glomerular filtration rate (*GFR).*

***Mechanism:***

The kidney's ability to perform many of its functions depends on the (3) fundamental functions of filtration, reabsorption, and secretion, whose sum is renal excretion. That is:

Urinary excretion rate = Filtration rate - Reabsorption rate + Secretion rate.[124]

Filtration:

The blood is filtered by nephrons, the functional units of the kidney. Each nephron begins in a renal corpuscle, which is composed of a glomerulus enclosed in a Bowman's capsule. Cells, proteins, and other large molecules are filtered out of the glomerulus by a process of ultrafiltration, leaving an ultrafiltrate that resembles plasma (except that the ultrafiltrate has negligible plasma proteins) to enter Bowman's space. Filtration is driven by Starling forces.

The ultrafiltrate is passed through, in turn, the proximal convoluted tubule, the loop of Henle, the distal convoluted tubule, and a series of collecting ducts to form urine.

***Reabsorption:***

Tubular reabsorption is the process by which solutes and water are removed from the tubular fluid and transported into the blood. It is called reabsorption (and not absorption) because these substances have already been absorbed once (particularly in the intestines).

Reabsorption is (2) steps process beginning with the active or passive extraction of substances from the tubule fluid into the renal interstitium (the connective tissue that surrounds the nephrons), and then the transport of these substances from the interstitium into the bloodstream. These transport processes are driven by Starling forces, diffusion, and active transport.

***Indirect reabsorption:***

In some cases, reabsorption is indirect. For example, bicarbonate *($HCO_3$-)* does not have a transporter, so its reabsorption involves a series of reactions in the tubule lumen and tubular epithelium. It begins with the active secretion of a hydrogen ion *(H+)* into the tubule fluid via a $Na^+/H^+$ exchanger:

- In the lumen.
- The $H^+$ combines with $HCO_3^-$ to form carbonic acid *($H_2CO_3$).*
- Luminal carbonic anhydrase enzymatically converts $H_2CO_3$ into $H_2O$ and $CO_2$.
- $CO_2$ freely diffuses into the cell.
- In the epithelial cell
- Cytoplasmic carbonic anhydrase converts the $CO_2$ and $H_2O$ (which is abundant in the cell) into $H_2CO_3$
- $H_2CO_3$ readily dissociates into $H^+$ and $HCO_3^-$
- $HCO_3^-$ is facilitated out of the cell's basolateral membrane.

***2.11.3: Renal Function:***

The kidney participates in whole-body haemeostasis, regulating acid-base balance, electrolyte concentrations, extracellular fluid volume, and regulation of blood pressure. The kidney accomplishes these homeostatic functions both independently and in concert with other organs, particularly those of the endocrine system. Various endocrine hormones coordinate these endocrine

functions; these include renin, angiotensin (II), aldosterone, antidiuretic hormone, and atrial natriuretic peptide, among others.

Many of the kidney's functions are accomplished by relatively simple mechanisms of filtration, reabsorption, and secretion, which take place in the nephron. Filtration, which takes place at the renal corpuscle, is the process by which cells and large proteins are filtered from the blood to make an ultrafiltrate that eventually becomes urine. The kidney generate (180) liters of filtrate a day, while reabsorbing a large percentage, allowing for the generation of only approximately (2) liters of urine. Reabsorption is the transport of molecules from this ultrafiltrate and into the blood. Secretion is the reverse process, in which molecules are transported in the opposite direction, from the blood into the urine.

***2.11.3.1: Excretion of wastes:***

The kidneys excrete a variety of waste products produced by metabolism. These include the nitrogenous wastes called "urea", from protein catabolism, as well as uric acid, from nucleic acid metabolism. Formation of urine is also the function of the kidney.

***2.11.3.2: Acid-base homeostasis:***

Two organ systems, the kidneys and lungs, maintain acid-base haemostasis, which is the maintenance of *pH* around a relatively stable value. The lungs contribute to acid-base homeostasis by regulating bicarbonate *($HCO_3^-$) concentration*. The kidneys have two very important roles in maintaining the acid-base balance: to reabsorb bicarbonate from urine, and to excrete hydrogen ions into urine

***2.11.3.3: Osmolality regulation:***

Any significant rise in plasma osmolality is detected by the hypothalamus, which communicates directly with the posterior pituitary gland.

An increase in osmolality causes the gland to secrete antidiuretic hormone *(ADH)*, resulting in water reabsorption by the kidney and an increase in urine concentration. The two factors work together to return the plasma osmolality to its normal levels.

*ADH* binds to principal cells in the collecting duct that translocate aquaporins to the membrane, allowing water to leave the normally impermeable membrane and be reabsorbed into the body by the vasa recta, thus increasing the plasma volume of the body.

There are two systems that create a hyperosmotic medulla and thus increase the body plasma volume: Urea recycling and the 'single effect.' Urea is usually excreted as a waste product from the kidneys. However, when plasma blood volume is low and *ADH* is released the aquaporins that are opened are also permeable to urea. This allows urea to leave the collecting duct into the medulla creating a hyperosmotic solution that 'attracts' water. Urea can then re-enter the nephron and be excreted or recycled again depending on whether *ADH* is still present or not.

The 'Single effect' describes the fact that the ascending thick limb of the loop of Henle is not permeable to water but is permeable to *NaCl*. This allows for a countercurrent exchange system whereby the medulla becomes increasingly concentrated, but at the same time setting up an osmotic gradient for water to follow should the aquaporins of the collecting duct be opened by *ADH*.

### *2.11.3.4: Blood pressure regulation:*

Main articles: Blood pressure regulation and Renin-angiotensin system

Long-term regulation of blood pressure predominantly depends upon the kidney. This primarily occurs through maintenance of the extracellular fluid compartment, the size of which depends on the plasma sodium concentration. Although the kidney cannot directly sense blood pressure, changes in the

delivery of sodium and chloride to the distal part of the nephron alter the kidney's secretion of the enzyme renin. When the extracellular fluid compartment is expanded and blood pressure is high, the delivery of these ions is increased and renin secretion is decreased. Similarly, when the extracellular fluid compartment is contracted and blood pressure is low, sodium and chloride delivery is decreased and renin secretion is increased in response.

Renin is the first in a series of important chemical messengers that comprise the renin-angiotensin system. Changes in renin ultimately alter the output of this system, principally the hormones angiotensin (II) and aldosterone. Each hormone acts via multiple mechanisms, but both increase the kidney's absorption of sodium chloride, thereby expanding the extracellular fluid compartment and raising blood pressure. When renin levels are elevated, the concentrations of angiotensin (II) and aldosterone increase, leading to increased sodium chloride reabsorption, expansion of the extracellular fluid compartment, and an increase in blood pressure. Conversely, when renin levels are low, angiotensin (II) and aldosterone levels decrease, contracting the extracellular fluid compartment, and decreasing blood pressure.

***2.11.3.5: Hormone secretion:***

The kidneys secrete a variety of hormones, including erythropoietin, and the enzyme renin. Erythropoietin is released in response to hypoxia (low levels of oxygen at tissue level) in the renal circulation. It stimulates erythropoiesis (production of red blood cells) in the bone marrow. Calcitriol, the activated form of vitamin D, promotes intestinal absorption of calcium and the renal reabsorption of phosphate. Part of the renin-angiotensin-aldosterone system, renin is an enzyme involved in the regulation of aldosterone levels.

### *2.11.3.6: Development:*

The mammalian kidney develops from intermediate mesoderm. Kidney development, also called nephrogenesis, proceeds through a series of three successive phases, each marked by the development of a more advanced pair of kidneys: the pronephros, mesonephros, and metanephros. [116]

### *2.11.3.7: Evolutionary adaptation:*

Kidneys of various animals show evidence of evolutionary adaptation and have long been studied in ecophysiology and comparative physiology. Kidney morphology, often indexed as the relative medullary thickness, is associated with habitat aridity among species of mammals.[117]

### *2.11.4: Renal Function Tests (RFTs):*

### *2.11.4.1: Blood Urea:*

Urea is the waste product of the degradation of amino acids into $CO_2$ and ammonia. Urea is synthesized in the liver and transported through blood to the kidney, where it is filtered through the glomerulus. Almost half of the urea is reabsorbed back into the blood by passive transport in the nephron tubule. Azotaemia may indicate renal disease or a nonrenal disorder that causes a secondary increase of blood urea as a consequence of disease.

The reference range for the *BUN*-to-c*reatinin*e ratio is (10:1) to (20:1). Increases in the ratio may be caused by prerenal, renal, and postrenal factors. Prerenal changes include variation of protein intake and dehydration. Renal disorders that affect the *BUN*-to-creatinine ratio include renal failure and glomerular damage.

The **blood urea nitrogen** *(BUN)* test is a measure of the amount of nitrogen in the blood in the form of urea, and a measurement of renal function. Urea is a by- product from metabolism of proteins by the liver and is removed from the blood by the kidneys.

***Interpretation:***

The most common cause of an elevated *BUN*, azotaemia, is poor kidney function, although a serum creatinine level is a somewhat more specific measure of renal function (see also renal function). A greatly elevated *BUN* (>60 mg/dL) generally indicates a moderate-to-severe degree of renal failure. Impaired renal excretion of urea may be due to temporary conditions such as dehydration or shock, or may be due to either acute or chronic disease of the kidneys themselves. An elevated *BUN* in the setting of a relatively normal creatinine may reflect a physiological response to a relative decrease of blood flow to the kidney (as seen in heart failure or dehydration) without indicating any true injury to the kidney. However, an isolated elevation of *BUN* may also reflect excessive formation of urea without any compromise to the kidneys.

Increased production of urea is seen in cases of moderate or heavy bleeding in the upper gastrointestinal tract (e.g. from ulcers). The nitrogenous compounds from the blood are resorbed as they pass through the rest of the GI tract and then broken down to urea by the liver. Enhanced metabolism of proteins will also increase urea production, as may be seen with high protein diets, patients on total parenteral nutrition, steroid use, burns, or fevers.

When the ratio of *BUN* to creatinine *(BUN: Cr)* is greater than (20), the patient is suspected of having prerenal azotemia. This means that the pathologic process is unlikely to be due to intrinsic kidney damage.

A low *BUN* usually has little significance, but its causes include liver problems, malnutrition (insufficient dietary protein), or excessive alcohol consumption. Overhydration from intravenous fluids can result in a low *BUN*. Normal changes in renal blood flow during pregnancy will also lower *BUN*.

Urea itself is not toxic. This was demonstrated by Johnson *et al.* by adding large amounts of urea to the dialysate of haemodialysis patients for several months and finding no ill effects.[119] However, *BUN* is a marker for other nitrogenous waste. Thus, when renal failure leads to a buildup of urea and

other nitrogenous wastes (uraemia), an individual may suffer neurological disturbances such as altered cognitive function (encephalopathy), impaired taste (dysgeusia) or loss of appetite (anorexia). The individual may also suffer from nausea and vomiting, or bleeding from dysfunctional platelets. Prolonged periods of severe uraemia may result in the skin taking on a sallow, yellowish discoloration or even forming frank urea crystals ("uraemic frost") on the skin.

Because multiple variables can interfere with the interpretation of a *BUN* value, *GFR* and creatinine clearance are more accurate markers of kidney function. Age, sex, and weight will alter the "normal" range for each individual, including race. In renal failure or chronic kidney disease *(CKD)*, *BUN* will only be elevated outside "normal" when more than (60) % of kidney cells are no longer functioning. Hence, more accurate measures of renal function are generally preferred to assess the clearance for purposes of medication dosing.

### *2.11.4.2: Serum Creatinine:*

***Creatinine Metabolism:***

Creatine is synthesized in the liver, pancreas, and kidneys from the amino acids arginine, glycine, and methionine. Creatine is transported through the circulatory system to muscle, brain, and other organs, where it is converted to phosphocreatine and acts as an energy reservoir much like *ATP*. Creatinine is produced as a waste product of creatine and phosphocreatine. Because much of the creatinine is produced in muscle, the amount of creatinine that is measured in blood is proportional to the patient's lean muscle mass. The waste product, creatinine, enters the blood supply, where it is removed through the kidneys.

**Creatinine** is a break-down product of creatine phosphate in muscle, and is usually produced at a fairly constant rate by the body (depending on muscle mass).

In chemical terms, creatinine is a spontaneously formed cyclic derivative of creatine. Creatinine is chiefly filtered out of the blood by the kidneys

(glomerular filtration and proximal tubular secretion). There is little-to-no tubular reabsorption of creatinine. If the filtering of the kidney is deficient, creatinine blood levels rise. Therefore, creatinine levels in blood and urine may be used to calculate the creatinine clearance *(CrCl),* which reflects the glomerular filtration rate (*GFR).*

The *GFR* is clinically important because it is a measurement of renal function. However, in cases of severe renal dysfunction, the creatinine clearance rate will be "overestimated" because the active secretion of creatinine will account for a larger fraction of the total creatinine cleared. Ketoacids, cimetidine and trimethoprim reduce creatinine tubular secretion and therefore increase the accuracy of the *GFR* estimate, particularly in severe renal dysfunction. (In the absence of secretion, creatinine behaves like inulin.)

A more complete estimation of renal function can be made when interpreting the blood (plasma) concentration of creatinine along with that of urea. *BUN-to-creatinine ratio* (the ratio of blood urea nitrogen to creatinine) can indicate other problems besides those intrinsic to the kidney; for example, a urea level raised out of proportion to the creatinine may indicate a pre-renal problem such as volume depletion.

Men generally tend to have higher levels of creatinine because they have more skeletal muscle mass than women. Vegetarians have been shown to have lower creatinine levels.[124)]

Measuring serum creatinine is a simple test and it is the most commonly used indicator of renal function.

A rise in blood creatinine level is observed only with marked damage to functioning nephrons. Therefore, this test is not suitable for detecting early-stage kidney disease. A better estimation of kidney function is given by the creatinine clearance (CrCl) test. Creatinine clearance can be accurately calculated using serum creatinine concentration and some or all of the following variables: sex, age, weight, and race, as suggested by the American Diabetes

Association without a (24)-hours urine collection, [118] some laboratories will calculate the CrCl if written on the pathology request form, and the necessary age, sex, and weight are included in the patient information.

A current concern in late 2010 relates to the adoption of a new analytical methodology, and a possible impact this may have in clinical medicine. All clinical laboratories in the *US* will soon use a new standardized Isotope Dilution Mass Spectrometry *(IDMS)* method to measure serum creatinine. *IDMS* appears to give lower values compared to older methods when the serum creatinine values are relatively low, for example (0.7) mg/dL. The *IDMS* method would result in a comparative overestimation of the corresponding calculated Glomerular Filtration Rate (*GFR*) in some patients with normal renal function. A few medicines are dosed even in normal renal function on that derived *GFR.* The dose, unless further modified, could now be higher than desired, potentially causing increased drug-related toxicity. To counter the effect of changing to *IDMS,* new *FDA* guidelines have suggested limiting doses to specified maximums with carboplatin, a chemotherapy drug.[119]

***Urine creatinine:***

Creatinine concentration is also checked during standard urine drug tests. High creatinine levels indicate a pure test, whereas low amounts of creatinine in the urine either indicate a manipulated test, either through the addition of water in the sample or by drinking excessive amounts of water, or low creatinine may simply be due to factors such as diet, medication, or other factors besides those of conscious urine manipulation.

It should be noted that diluted samples may not always be due to a conscious effort of subversion and diluted samples cannot be proved to be intentional, but are only assumed to be. Random urine creatinine levels have no standard reference ranges. They are usually used with other tests to reference levels of other substances measured in the urine. Diuretics, such as coffee and

tea, cause more frequent urination, thus potently decreasing creatinine levels. A decrease in muscle mass will also cause a lower reading of creatinine, as will pregnancy. [119]

***Interpretation:***

In the United States, creatinine is typically reported in mg/dL, whereas, in Canada and a few European countries, μmol/litre may be used. (1) mg/dL of creatinine is (88.4) μmol/L.

The typical human reference ranges for serum creatinine are (0.5) to (1.0) mg/dL about (45-90) μmol/L for women and (0.7 to 1.2) mg/dL (60-110 μmol/L) for men. While a baseline (medicine) serum creatinine of (2.0) mg/dL (150) μmol/L may indicate normal kidney function in a male body builder, a serum creatinine of (1.2) mg/dL (110) μm/L) can indicate significant renal disease in an elderly female. For male reference range are (60-120) μmol/L and for female it is (50-110) μmol/L.

More important than absolute creatinine level is the trend of serum creatinine levels over time.

Creatinine levels may increase when *ACE inhibitors (ACEI)* or angiotensin II receptor antagonists (or angiotensin receptor blockers, *ARBs*) are taken. Using both *ACEI & ARB* concomitantly will increase creatinine levels to a greater degree than either of the two drugs would individually. An increase of (<30) % is to be expected with *ACEI or ARB* use. [119]

***BUN-to-Creatinine ratio:***

In medicine, the *BUN-to-creatinine* ratio is the ratio of two serum laboratory values, the blood urea nitrogen (*BUN) (mg/dL*) and serum creatinine (*mg/dL) (Cr).* Outside the United States, particularly in Canada and Europe, the truncated term urea is used (though it is still the same blood chemical) and the units are different *(mmol/L).* The units of creatinine are also different *(*μmol/L*),*

and this value is termed the *urea-to-creatinine ratio*. The ratio may be used to determine the cause of acute kidney injury.

The principle behind this ratio is the fact that both urea *(BUN)* and creatinine are freely filtered by the glomerulus; however urea reabsorbed by the tubules can be regulated (increased or decreased) whereas creatinine reabsorption remains the same (minimal reabsorption).

***Interpretation:***

***Serum Ratios:***

An elevated *BUN: Cr* due to a low or low-normal creatinine and a *BUN* within the reference range are unlikely to be of clinical significance.

***Specific causes of elevation:***

***Acute Kidney Injury (previously termed Acute Renal Failure):***

The ratio is predictive of prerenal injury when *BUN: Cr* exceeds 20[124] or when *urea: Cr* exceeds (0.1) and urea is greater than (10) [118] in prerenal injury, urea increases disproportionately to creatinine due to enhanced proximal tubular reabsorption.

***Gastrointestinal bleeding***

The ratio is useful for the diagnosis of bleeding from the gastrointestinal (GI) tract in patients who do not present with overt vomiting of blood. [119] in children, a *BUN: Cr* ratio of (30) or greater has a sensitivity of (68.8) % and a specificity of (98) % for upper gastrointestinal bleeding.[120] *BUN* level increases in upper GI bleeding because patients become prerenal, secondary to blood loss which decreases blood flow to the kidney.

A common misconception is that the ratio is elevated because of amino acid digestion. Since blood, which consists largely of the protein haemoglobin, is broken down by digestive enzymes of the upper GI tract into amino acids, which are then reabsorbed in the GI tract and broken down into urea. However,

elevated *BUN: Cr* ratios are not observed when other high protein loads (e.g., steak) are consumed.

***Advanced age:***

Because of decreased muscle mass, elderly patients may have an elevated *BUN: Cr* at baseline. [121]

### *2.11.4.4: Serum Electrolyte:*

The four major electrolytes are sodium ($Na^+$), potassium ($K^+$), chloride ($Cl^-$) and bicarbonate ($HCO_3^-$). Sodium and potassium are **cations**, with sodium in greater concentration extracellularly and potassium in greater concentration intracellularly. Chloride and bicarbonate are **anions**. Control of cation concentration is maintained by the *Na-K-ATPase pump*.

#### *2.11.4.4.1: Sodium:*

Because of the high concentration of sodium in extracellular fluid, such as plasma water, sodium plays a major role in maintaining **osmotic pressure**. Sodium levels in body fluids are maintained by renal reabsorption in the proximal convoluted tubules, based on a sodium threshold. Sodium balance is also maintained by the influence of the hormone aldosterone on the distal convoluted tubules, as a consequence of changes in blood volume and blood pressure. Extremely high or low sodium concentrations in plasma will cause severe osmotic pressure changes that can induce serious consequences to several organs. The most immediate effect is swelling on the brain and potential coma. (125)

#### *2.11.4.4.2: Potassium:*

Potassium, the major intracellular cation, is also controlled by the *Na-K-ATPase pump*. Potassium maintains cardiac rhythm and contributes to neuromuscular conduction. Imbalances in potassium level, as indicated by

hyperkalaemia or hypokalaemia, will cause cardiac arrhythmias and neuromuscular weakness.

***2.11.4.4.3: Chloride:***

Chloride is one of the major extracellular anions that helps maintain electrical neutrality with sodium. Chloride contributes to the maintenance of acid-base balance by participating in the isohydric shift. The isohydric shift describes the maintenance of hydrogen ion concentration as shifting of hydrogen ion occurs between fluid spaces. The shift refers to the buffering of $H^+$ with $HCO3^-$ and other buffers intracellularly and movement to extracellular fluid spaces. $H^+$ produced from dissolved $CO_2$ and carbonic acid is buffered in plasma by deoxyhaemoglobin and plasma buffers. Thus, the $H^+$ and $CO_2$ concentrations are similar in venous and arterial blood due to their flow into and out of cells, including the capillary, venous, and arterial extracellular fluids. The chloride shift is the movement of $Cl^-$ ions opposite to bicarbonate ions during acid-base compensation and, thus, maintains electrical neutrality as due to increased protonation of proteins and *haemoglobin*.

***2.11.4.4.4: Bicarbonate:***

Bicarbonate ion is produced from carbon dioxide as it reacts with the water in the plasma. It forms from the reaction:

$CO_2 + H_2O \leftrightarrow\square H_2CO_3 \leftrightarrow\square H^+ + HCO_3^-$

Bicarbonate is the major component of the extracellular buffer system, and is Controlled by renal tubular cells and erythrocytes, the previously described metabolic component of the buffer system. While total $CO_2$ $(tCO_2)$ includes many components, the majority of plasma $CO_2$ exists in the form of bicarbonate ion. Some bicarbonate is found in cells (intracellulary), where it maintains electrical neutrality in conjunction with potassium. Bicarbonate may move to extracellular fluid in order to buffer $H^+$, forming $H_2CO_3$. Carbonic acid is a weak acid that will act as a buffer and not contribute to the *pH* of the body

fluids. Dissolved carbon dioxide gas is measured as $PCO_2$ by blood gas analyzers and contributes to $H_2CO_3$ levels. Dissolved $CO_2$ and $PCO_2$ make up a small percentage of carbon dioxide forms. A minute amount of carbon dioxide in plasma is bound to carrier proteins. [126].

***Abnormal Sodium Levels:***

Hypernatraemia is caused by renal and nonrenal disorders. A common nonrenal cause is hypotonic dehydration from severe diarrhoea, extensive burns, or excessive sweating without proper fluid replacement. Infants, the elderly and other patients not able to ingest sufficient amounts of water, and who are not properly hydrated, will also experience hypernatraemia. Renal loss of water, such as in nephrogenic diabetes insipidus, can also cause hypernatraemia. Serum osmolality and urinary sodium levels can help to differentiate renal loss of water versus nonrenal causes. [125]

**Hyponatraemia** is caused by renal and nonrenal causes also. Salt-losing renal Nephritis, renal tubular acidosis, or syndrome of inappropriate antidiuretic hormone secretion *(SIADH)* are common causes of renal loss of sodium and may be evaluated by testing for the presence of excess urinary sodium and hyperosmolar urine. Certain diuretics, such as thiazine, can cause renal loss of sodium. Increased urine sodium levels usually indicate sodium loss. Chronic renal failure can cause water overload due to inability to regulate water and results in hyponatraemia, while nephritic syndrome can cause fluid imbalances and edema with resulting hyponatraemia. Urine sodium levels are usually normal or decreased in hyponatraemia due to edema. Nonrenal causes of hyponatraemia include psychogenic water overload, cellular shift changes from acidosis, and oedema secondary to cirrhosis or congestive heart failure. [125]

***Abnormal Potassium Levels:***

Hyperkalaemia may be caused by decreased renal excretion in acute or chronic renal failure, certain diuretics, or hypoaldosteronism or hypocortisolism.

Hyperkalaemia may also be caused by ion shift, such as the ion shift that is seen in cases of diabetic ketoacidosis or other metabolic acidosis, leukaemia, excessive muscle activity, or haemolysis. Finally, hyperkalaemia is associated with iatrogenic causes of excessive intravenous or oral therapy.

**Hypokalaemia** is caused by renal loss such as renal tubular acidosis, hyperaldosteronism, hypocortisolism, and certain diuretics. Potassium can also be decreased due to gastrointestinal dietary deficit or loss from severe vomiting, diarrhea, nasogastric suctioning, laxatives, and malabsorption. A cellular shift in cases of insulin overdose and alkalosis can also cause hypokalaemia. (127)

***Renal Impact on Water and Electrolytes:***

Filtration removes the majority of electrolytes, nutrients, and waste products and some water in the glomerulus. Reabsorption of much of the sodium, chloride, potassium, minerals, amino acids, glucose, and the like occurs in the proximal convoluted tubule by passive transport, facilitated diffusion, and active transport. More electrolytes and water are reabsorbed in the loop of Henle. Under hormonal influence of antidiuretic hormone, parathyroid hormone, and aldosterone, reabsorption occurs in the distal convoluted and collecting tubules. (128)

***Hormonal and Renal Control of Electrolytes and minerals:***

Parathyroid hormone regulates mineral levels by causing renal reabsorption of calcium and magnesium in exchange for phosphorus. More phosphorus is excreted in the urine, and is used in the phosphate buffer system, while calcium is reclaimed to the plasma. Antidiuretic hormone *(ADH*), also known as vasopressin, is secreted by the hypophysis in response to osmoreceptors in the brain. *ADH* stimulates the renal collecting ducts to reabsorb water, thus conserving more water in times of water deprivation.

Epinephrine is a catecholamine that raises blood pressure by constricting

blood vessels but does not impact upon electrolyte or mineral levels in extracellular fluids. Aldosterone, a mineralocorticoid secreted by the adrenal cortex, induces the distal convoluted tubules to reabsorb sodium and chloride in exchange for potassium. Since these hormones function in an integrated fashion, they help to maintain homeostasis of minerals and electrolytes by finishing the reabsorption and secretion phase within the kidney.

Electrolyte disturbances according to various disease states are listed below:

1. Dehydration is associated with elevated plasma sodium, plasma osmolality, *BUN*, total serum protein, and haematocrit. Although many dissolved particles in serum are relatively elevated, these are the most sensitive indicators of dehydration.
2. Acute renal failure causes elevated plasma potassium, magnesium, phosphorus, and creatinine and often decreased plasma calcium, sodium, and bicarbonate levels.
3. Cellular breakdown from haemolysis or trauma or in stored blood samples causes increased plasma potassium, magnesium, and phosphorus.
4. Treatment with insulin following hyperglycaemia will cause a drop in plasma potassium and phosphorus.
5. Overtreatment with electrolytes will cause imbalances. For example, overtreatment with potassium intravenous (IV) fluids or orally will cause hyperkalaemia, and overtreatment with sodium will cause hypernatraemia and hyperosmolality.
6. Overhydration with IV fluids will cause hyponatraemia, hypochloraemia, and lowered plasma osmolality. [113].

### *2.11.4.5: Cystatin C:*

Problems with creatinine (varying muscle mass, recent meat ingestion, etc.) have led to evaluation of alternative agents for estimation of *GFR*. One of

these is c*ystatin C,* a ubiquitous protein secreted by most cells in the body (it is an inhibitor of cysteine protease). Cystatin C is freely filtered at the glomerulus. After filtration, *Cystatin C* is reabsorbed and catabolized by the tubular epithelial cells, with only small amounts excreted in the urine. *Cystatin C* levels are therefore measured not in the urine, but in the bloodstream. Equations have been developed linking estimated *GFR* to serum *cystatin C* levels. Most recently, some proposed equations have combined (sex, age and race) adjusted cystatin C and creatinine. The most accurate is (sex, age and race) adjusted *cystatin C,* followed by (sex, age and race) adjusted creatinine and then *cystatin C* alone in slightly different with adjusted creatinine. [128]

In a study conducted by *Ahmed SH, Danish T, Faridi MM, Ahmed Aj, Fakhir S kKhan AS* to evaluate the Renal function in acute malaria in children they report a rise in urine protein (150 – 1500) mg/day was found in (12) patients out of (27) patients with *P.falciparum* infection(129) . Poly acryl amide gel electrophoresis revealed either an increase in low or high molecular weight proteins alone or an increase in both low and high molecular weight proteins. Serum urea and Creatinine were increase in five of these patients. In *P.vivax* infections eight out of (46) patients developed a proteinuria of up to (462) mg/day. The *authors* concluded that there was correlation between the appearance of proteinuria and fever and not its height. It is therefore unlikely that a rise in temperature is the only cause of proteinuria in malarial infection. The electrophoretic analysis of proteinuria indicated that in malarial infection, glomerular as well as tubular lesions may cause reversible proteinuria (129).

Narrow- bore granular cast was observed by *M.D.Mustafa* and *G.M.Elimari* in (111) patients out of (213) Sudanese patients with malaria. (130)

*Etim O.E* and his colleagues were reported a significant ($P<0.05$) decrease in the levels of $Na^{+}$, CI" and $HCO^{-}_{3}$, while urea, creatinine potassium

and anion gap increased significantly in malaria patients compared to those of normal controls. [131]

### *2.12: Haematological change:*

Malaria has long featured prominently in the grey area between parasitological and haematology. In a classical European textbook of haematology published in the 1930s was defined as a "typical blood disease" characterized by fever, anaemia and Splenomegaly, [132] it is currently considered a typical example of a haemolytic anaemia in more recent haematology textbooks, due to an acquired extra-corpuscular cause. As parasites of the blood for the majority of their complex life cycle, they expectedly induce haematological alterations. The haematological abnormalities that have been reported to invariably accompany infection with malaria include anaemia, thrombocytopaenia, Splenomegaly, and mild-to-moderate atypical lymphocytosis and rarely disseminated intravascular coagulation (*DIC)* [130, 131]. There have also been reports of leucopaenia and leucocytosis [22]. Other hematological reactions to malaria that have been reported include neutropaenia, eosinophilia, neutrophilia and monocytosis [132-134]. Some controversies appear to exist however. Many of the studies on the haematological abnormalities have been conducted in endemic countries, some only in children and some only in severe malaria patients. Relatively few studies have been done among non-immune or semi-immune travelers returning from endaemic areas or patients returning from their endemic countries [135].

#### *2.12.1: The platelets:*

Platelets are not true cells. They are actually fragments of large bone marrow cells called megakaryocytes. They are small sized diskettes about (3-4) μm in diameter, that on the whole amount to a mean measure of roughly (300,000) cells per μl of blood. Individually, each features a cell of (7) fl, and a mean surface area of $8\mu m^2$. In aggregate, blood platelets display a larger total

volume and surface area than the aggregate of all other leukocyte subtypes taken together. Platelets are often classed as blood cells, and undoubtedly, play an eminent role in hemostasis and thrombosis. However, a recent research showed that platelets help to fight infections, by means that clearly exceed an exclusive function as mere players in the primary physiological processes. On activation, platelets exhibit the ability to release considerable quantities of secretory products and express a multitude of immune receptors on their membrane. They are characterized by an open canalicular system, which contributes to the engulfment and/or filtration of serum components, pathogens or antigens. The identification of chemokines in blood platelets has strengthened the view of these cells as participants in immune host defense. Platelet chemokines, representing pre-stored and rapidly releasable proteins, may play a major role as first-line inflammatory mediators. This is evident from their capability to recruit early inflammatory cells such as neutrophil granulocytes and monocytes and even to exhibit direct antimicrobial activity. However, insight is growing that platelet chemokines may also be long-term regulators, e.g., by activating T lymphocytes. Regarding parasitic infections, blood platelets are suggested to have a role in destroying parasites. This finding may have an implication in treatment of parasites {136}.

### *2.12.2: Platelets count:*

Is used to detect a low or high number of platelets in the blood .the test is included in a complete blood count (*CBC)*.and the test done either manually using a haemocytometer or by placing blood in an automated platelets analyzer using electrical impedance, such as a coulter counter . The normal range is 150,000 - 450,000per cubic millimeter {137}, .increase in number of platelets called thrombocytosis and decrease in number called thrombocytopaenia.

Thrombocytopaenia occurs due to:

1. Decrease bone marrow production (hypoplasia, replacement of bone marrow cellular due to malignant haemopoeitic cell, infective erythropoiesis).
2. Increase consumption of platelets (immune thrombocytopaenia *ITP*, drug induced immune thrombocytopenia, infection, post transfusion purpura, disseminated intravascular coagulation *DIC*, thrombotic thrombocytopaenic purpura *TTP*, haemolytic uraemic syndrome).
3. Increase splenic pooling.
4. Dillutional loss.
5. Technical errors.
6. Other causes such as systemic lupus erythromatus *SLE*, sepsis, and liver disease, malignant causes, snake venom.

**Thrombocytosis occur due to:**

1. Primary thrombocytosis or essential thrombocythaemia (a form of myeloproliferative disease).
2. Secondary thrombocytosis (over production of pro-inflammatory cytokines such as IL1, IL6, IL11).

### *2.12.3: Mean platelets volume (MPV*):

Is calculated measurement of the average size of platelets found in blood and is typically included in blood tests as part of the *CBC*. Since the average platelet size is larger when the body is producing increased numbers of platelets, the *MPV* test results can be used to make inferences about platelet production in bone marrow or platelet destruction problems. *MPV* is higher when there is destruction of platelets. This may be seen as in inflammatory bowel disease, and in *ITP*, and in myeloproliferative diseases and Bernard-Soulier syndrome. It may also be related to pre-eclampsia, and recovery from transient hyperplasia. Abnormally low *MPV* values correlate primarily with thrombocytopaenia when it is due to impaired production as in aplastic anaemia. A typical range of

platelet volumes is (9.7–12.8) fL (femtoliter), equivalent to spheres (2.65 to 2.9) µm in diameter. Normal range is given as (7.5-11.5) fL [{138}].

### *2.12.4: Platelets distribution width (PDW):*

It's the width of the size distribution curve in femtoliter (fl). Reflects the variability in the platelet size, and it is therefore increased in the presence of platelet anisocytosis. A high *PDW* a sign of active platelets release .The *PDW* median is (13.3) %with reference range of (10-17.9) % [{139}].Haematological changes are some of the most common complications in malaria as the changes involve platelets count and their indices. Thrombocytopaenia in malaria has been reported, but the extent of these alterations varies with the level of malaria, endemicity, background haemoglobinopathy, nutritional status, demographic factors, and malaria immunity [{140}].

### *2.12.5: Platelets and parasite:*

Changes that occur during platelet activation affect not only the membrane but also their internal structures. Activation causes platelets' micro tubular de-polymerization and actin polymerization, which leads to a change in their shape and formation of pseudopodia. Degranulation that occurs due to the fusion of intra-platelet granular membranes with the cytoplasmic membrane is a direct sequel to activation. The release reaction is strictly correlated with the appearance of a platelet activation dependent granule external membrane glycoprotein on platelet surface, known as *CD62P* cell adhesion molecule *(CAM).* This glycoprotein is found on the surface of activated platelets and activated endothelial cells, and is also known as P-selectin. Blood platelets unfold themselves revealing receptors for adhesion molecules, particularly for fibrinogen. Amplification of platelet aggregates and generation of thrombin on phospholipids surfaces of blood platelets can be observed [{141}]. Structural and functional changes in blood platelets that occur during their activation include the presence of micro platelets, micro platelets are fragments of platelets or their

cellular membranes that originate due to disintegration of activated platelets; or are fragments of cytoplasm of megakaryocytes formed during enhanced thrombocytopoiesis. They are the first to be released during adhesion to the vascular wall, and clinical disorders present at that time are associated with their increased percentage. Platelet stimulation may occur as a result of direct contact with a parasite, increase IgG and/or IgE concentration, the presence of complement components, C-reactive protein or lymphokines. Stimulated blood platelets reveal cytotoxic activity through release of various inflammatory mediators. Cytotoxic properties of blood platelets are induced by cytokines such as *interferon gamma (INF-γ), tumor necrosis factor alpha (TNF-α*) and *interleukin 6 (IL-6).* The mechanism of adhesion between platelets and parasites has not been fully elucidated but it is known to depend on platelet surface receptors. Activated platelets also show phagocytic activity and cooperation with other cells of immunologic system [{142}]. *Eze Evelyn M, F. C. Ezeiruaku*, and *D. C. Ukaji*, observed trend of decreasing platelet count with increasing levels of parasitaemia in their study in 201. [(177)]

### *2.13: Diagnostic Efficiency*

Parameters of diagnostic efficiency are intended to quantify how useful a test is for a given disease or condition. It includes sensitivity, specificity, and predictive values. [(143)]

### *2.13.1: Sensitivity:*

Refers to the proportion of individuals with that disease who test positively with the test. Sensitivity can be calculated from simple ratios Patients with a condition that are correctly classified by a test to have the condition is called true positives *(TPs).* Patients with the condition who are classified by the test as not having the condition are called false negatives *(FNs).*

***Sensitivity %= (TP) / (TP + FN)***

### *2.13.2: Specificity*

Diagnostic specificity is defined as the proportion of individuals without a condition who have a negative test for that condition.

***Specificity= (TN) / (TN + FP)***

### *2.13.3: Predictive value of a positive test:*

Refers to the probability of an individual having the disease if the result is abnormal ("positive" for the condition).

### *2.13.3: Predictive value of a negative test:*

Refers to the probability that a patient does not have a disease if a result is within the reference range (test is negative for the disease). [143] Predictive values are also calculated using ratios of true positives, true negatives, false positives, and false negatives (FNs) as follows:

***PPV = (TP)/ (TP + FP NPV = (TN) / (TN + FN)***. [143]

Carol J et al report that sensitivity of *Optimal* (Flow Inc., Portland), to detect *P. falciparum* malaria were (99) % and specificity of (100) % for malaria diagnosis when compared to those obtained by reading (100) fields of traditional Giemsa-stained thick-smear blood films for the detection of *P. vivax* and *P.falciparum* malaria. [144]

# 3: Material and Methods

### *3.1: Study area:*

Shendi locality, River Nile State, Sudan, which located north of Khartoum, about (176) km. The total areas of Shendi locality are about (1496) $km^2$. Shendi population about (55.516) persons male (48.7) % female (51.3) % (2007), most of them are farmers.

***3.2: Study design:*** This study was prospective case control study.

### *3.3: Study population & Sample size:*

(153) samples, ages (12 to 65) years, mean age (31.3) years, (92) male (60.1%) and (61) female (39.9%), were studied in the period from November 2010 to Oct 2014.All patients had a sexual form of w malaria parasite confirmed by thin and thick blood films. (122) patients had *P.falciparum* (79.7%) and (31) *P.vivax* (20.3%). Fifty age and sex matched control (using same percentage ratios as patient group) were studied. All patients and controls were free from any previous conditions which could affect the parameters under study (Renal disease, liver disease, hypertension, *D.M*, pregnancy and history of platelets disorders) Sample size was calculated by using $N=Z^2$ (P (1-P)/$e^2$, where N=sample number, $Z^2$ = level of confidence on the sample, P= proportion probability of the aim variable falling in the group in which you are interested, and e= the accepted margin of error.

### *3.4: Inclusion criteria:*

a- Case group: Sudanese peoples with malaria parasite infection.

b- Control group: Sudanese complete healthy volunteers.

### *3.5: Exclusion criteria:*

Any patient with other disease which had effect on parameters under study (Renal disease, liver disease, hypertension, *D.M*, pregnancy, platelets disorder), and patient had given antimalarial treatment been ruled out.

### *3.6: Tools of data collection:*

A questionnaire was filled for each patient and control and proper examination was done for each.

### *3.7: Ethical consideration:*

This research was performed after the consent of all the people involved in it, and was taking into account the trust and strict confidentiality with respect to patients and information about them, and was scheduled, based on reliable information and reliable source. This study was identical for all human rights. During data collection from patients or relatives, verbal consent was obtained, and names and personal data were completely secured and transferred to codes to keep patients' identities private.

### *3.8: Sample collection:*

(5) ml of blood were collected by clean venepuncture using sterile disposable syringes, and immediately divided into *EDTA* blood container (whole blood) and plain (No anticoagulant) container which left to clot and after clot retraction, serum was separated by centrifugation and kept in a deep freezer for the next day. Each patient was requested to pass urine in a clean plastic container.

### *3.9: Data analysis and presentation:*

All data was analyzed using statistical package for the social sciences (SPSS) version *(15*). The mean was obtained and cross –tabulated test, independent T- test, Chi square test and Spearman correlation were used for comparison and presented in form of figures and tables. *P value* and odd ratio was obtained to assess the significant of the result.

### *3.10: Methods:*

#### *3.10.1: Detection of Malaria parasite:*

Thick and thin blood films were done. After drying the thin films were fixed with absolute methanol for (30) seconds. The films were stained with freshly prepared (10) % Giemsa stain by mixing 1ml of tock solution with 9ml of buffer (pH: 7.2). The slides were flushed with tap water and lift in the upright position to dry. The blood films were examined using (100) oil immersion lens. A positive smear was included with each new batch of working Giemsa stain for quality control. Parasite densities were assessed as parasite/thick film field. All slides were double-checked and only considered negative if no parasites were detected in (100) oil immersion lens.

**Parasites were counted and Parasitaemia graded as follow:**

1-10 parasite\ 100 field (+)

1-10 parasite\ 10 field (++)

1- 10 parasite\ 1 field (+++)

>10 parasite\ 1 field (++++).

Thin blood films were used for the identification of the plasmodium species.

***Principle of Optimal Malaria Test:***

It is a qualitative, membrane based immunoassay for the detection of *P.f, P.v, P.o and P. m* antigens in whole blood. The membrane is pre-coated with anti – *HRP* antibodies and anti aldolase antibodies. During testing, the whole blood specimen reacts with the dye conjugate, which has been pre-coated on the test strip. The mixture then migrates upward on the membrane by capillary action, reacts with anti Histidin rich protein II *(HPR- II* ) antibodies on the membrane on *P.f* test line region and with anti – aldolase antibodies on the membrane on Pan line region. The absence of colored lines in *P.f* region or Pan Line region indicates that the specimen does not contain *HPR-II* and/or plasmodium specific aldolase.

**Procedure:** The investigations of malaria using 'Optimal Rapid Malaria Dipstick Test was performed on venous blood sample drawn into *EDTA* tubes for all patients by transferring (10) µl of blood to *well-1* follow by adding three drops of buffer on *well-2*. The timer was started, after (5) minutes the result was interpreted.

### *3.10.2: Mindray Haematology Analyzer (Mindray bc-3000):*

**1. Principle:** blood cells can be broadly divided into three categories .red blood cells, White blood cells and platelets. The analyzer measures the number of cells and distinguishing between their types according to size using sheath flow *DC* detection. Electrical current is passed through a solution; this method measures the changes in electrical resistance that occurs when blood cells pass through detection aperture.

**2. Method:** the *EDTA* blood samples were aspirated into analyzer through a sample probe, and the counting was started automatically, the results were displayed on the screen within (20) second, the print key was pressed to print out the results.

### *3.10.3: Urine Examination (Routine Dipstick)*

The urine dipstick is a plastic strip to which paper tabs impregnated with chemical reagents have been affixed. The reagents in each are chromogenic. After timed development, the color on the paper segment is compared with a chart. Some reactions are highly specific. Others are sensitive to the presence of interfering substance or extremes of pH. Discoloration of the urine bilirubin or blood may obscure the color.[144]

Fresh urine samples were examined with test strips (Camphor 10) from (Healgen- USA) for pH, Nitrite, Protein, Glucose, Ketones, Urobilinogen, Bilirubin, Specific Gravity, Leukocyte and blood.

### *3.10.3.1: Urine pH:*

The pH test pads use indicator dyes that change color with *pH*. The physiologic urine *pH* ranges from (4.5 to 8). The determination is most accurate if done promptly, because growth of urea-splitting bacteria and loss of $CO_2$ raise the *pH*. In addition, bacterial metabolism of glucose may produce organic acids and lower pH.

### *3.10.3.2: Urine Protein:*

Protein measurement uses the protein error of indicators principle. The *pH* at which some indicators change color varies with the protein concentration of the bathing solution. Protein indicator strips are buffered at an acid *pH* near their colour change point. Wetting them with a protein containing specimen induces a colour change. The protein reaction may be scored from trace to 4+ or by concentration. Highly alkaline urine, especially after contamination with quaternary ammonium skin cleansers, may produce false-positive reactions.
Protein strips are highly sensitive to albumin but less so to globulins, hemoglobin or light chain. If light-chain proteinuria is suspected, more sensitive assays should be used. With acid precipitation test, an acid that denatures protein is added to the urine specimen and the density of the precipitate is related to the protein concentration. Urine that is negative by dipstick but positive with sulfosalicylic acid is highly suspicious for light chains. Tolbutamide, high-dose penicillin, sulfonamides, and radiographic-contrast agents may yield false-positive turbidimetric reactions. More sensitive and specific tests for light chains are preferred.

If the urine is very concentrated, the presence of a modest protein reaction is less likely to correspond to significant proteinuria in a 24-hour collection. Even so, it is unlikely that a (3+ or 4+) reaction would be seen solely because of a high urine concentration or conversely that the urine would be dilutes enough to give a negative reaction despite significant proteinuria. The

protein indicator used for routine dipstick analysis is not sensitive enough to detect microalbuminuria. [144]

### *3.10.3.3: Urine Blood:*

Reagent strips for blood rely on the peroxidase activity of haemoglobin to catalyze organic peroxide with subsequent oxidation of an indicator dye. Free hemoglobin produces a homogeneous color. Intact red cells cause punctate staining. False-positive reactions occur if the urine is contaminated with other oxidants such as povidone-iodine, hypochlorite or bacterial peroxidase. Ascorbate yields false-negative results. Myoglobin is also detected, because it has intrinsic peroxidase activity. A urine sample that is positive for blood by dipstick analysis but shows no red cells on microscopic examination is suspected for myoglobinuria or haemoglobinuria. Pink discoloration of serum may occur with haemolysis, but free myoglobin is seldom present in a concentration sufficient to change the color of plasma.

### *3.10.3.4: Urine Specific Gravity:*

Specific gravity reagent strips actually measure ionic strength using indicator dyes with ionic strength-dependent dissociation constants *(pKa).* They do not detect glucose or nonionic radiographic-contrast agents.

### *3.10.3.5: Urine Glucose:*

Modern dipstick reagent strips are specific for glucose. They rely on glucose oxidase to catalyze the formation of hydrogen peroxide, which reacts with peroxidase and a chromogen to produce a color change. High concentrations of ascorbate or ketoacids reduce test sensitivity. However, the degree of glycosuria occurring in diabetic ketoacidosis is sufficient to prevent false-negative results despite ketonuria. [144]

### *3.10.3.6: Urine Ketones:*

Ketone reagent strips depend on the development of a purple colour after acetoacetate reacts with nitroprusside. Some strips can also detect acetone, but none react with β-hydroxybutyrate. False-positive results may occur in patients who are taking levodopa or drugs such as captopril or mesna that contain free-sulfhydryl groups.

### *3.10.3.7: Urine Urobilinogen:*

Urobilinogen is a colorless pigment that is produced in the gut from metabolism of bilirubin. Some is excreted in feces and the rest is reabsorbed and excreted in the urine. In obstructive jaundice, bilirubin does not reach the bowel, and urinary excretion of urobilinogen is diminished. In other forms of jaundice, urobilinogen is increased. The urobilinogen test is based on the Ehrlich reaction, in which diethylaminobenzaldehyde reacts with urobilinogen in acid medium to produce a pink color. Sulfonamides may produce false-positive results and degradation of urobilinogen to urobilin may yield false-negative results. Better tests are available to diagnose obstructive jaundice. [144]

### *3.10.3.8: Urine Bilirubin:*

Bilirubin reagent strips rely on the chromogenic reaction of bilirubin with diazonium salts. Conjugated bilirubin is not normally present in the urine. False-positive results may be observed in patients receiving chlorpromazine or phenazopyridine. False-negative results occur in the presence of ascorbate.

### *3.10.3.9: Urine Nitrite:*

The nitrite screening test for bacteriuria relies on the ability of Gram-negative bacteria to convert urinary nitrate to nitrite, which activates chromogen. False-negative results occur when there is infection with Enterococcus or other organisms that do not produce nitrite, when ascorbate is

present or when urine has not been retained in the bladder long enough (approximately 4 hours) to permit sufficient production of nitrite from nitrate.

### *3.10.3.10: Urine Leukocytes:*

Granulocyte esterase can cleave pyrrole amino acid esters, producing free pyrrole that subsequently reacts with a chromogen. The test threshold is five to fifteen white blood cells *(WBCs)* per high-power field *(HPF).* False-negative results occur with glycosuria, high-specific gravity, cephalexin or tetracycline therapy or excessive oxalate excretion. Contamination with vaginal debris may yield a positive test result without true urinary tract infection.

## *3.10.4: Chemical analysis: (Automated ChemistryAnalyzer, Mindray BS 120):*

### *Principle of operation:*

After the tray is loaded with samples, a pipette aspirates a precisely measured aliquot of sample and discharges it into the reaction vessel; a measured volume of diluent rinses the pipette. Reagents are dispensed into the reaction vessel. After the solution is mixed (and incubated, if necessary), it is aspirated into a flow cell, where its absorbance is measured by a flow-through colorimeter.

The analyzer then calculates the analyte's chemical concentrations.

### *Operating Procedure:*

The sample tube has been loaded into the analyzer; reagents were already being stored in the instrument. The desired tests were programmed and the required tests (Total protein, Albumin, Total Bilirubin, Direct Bilirubin, Urea and Creatinine) were run, the results were displayed on-screen, printed out, stored in the analyzer's internal memory. [144]

### *3.10.4.1: Plasma total Protein:*

The Biuret Method for determination of plasma total protein was used in this study

**Principle:** proteins form a violet- blue complex with copper ions in alkaline solution. The absorbance of the complex is measured at (540) nm.

**Reagents:**

- Sodium hydroxide pellets (4) g/l
- Copper sulphate (3.0) g/l
- Potassium sodium tartrate tetrahydrate (9) g/l
- Potassium iodide (5) g/l

### *3.10.4.2: Plasma albumin:*

The *BCG* Method for estimation of plasma albumin was used in this study.

**Principle:**

Plasma albumin and buffered *BCG* (Bromocresol green) are allowed to bind at *pH* (4.2), and absorption of the *BCG*/Albumin complex is determined spectrometrically at (632) nm. Albumin act as a cation to bind the anionic dye. [145]

**Reagent:**

- BCG reagent in Succinate buffer:
- Succinic acid (5.6) g.
- Bromocresol green (58) mg
- Sodium azide (100) mg
- Distilled water (900) ml

### *3.10.4.3: Serum Bilirubin:*

The jendrassik Grof Method for estimation of serum bilirubin was used in this study.

**Principle:** Sulfanilic acid is diazotized by the nitrous acid produced from the reaction between sodium nitrite and hydrochloric acid. Both conjugated and unconjugated Bilirubin reacts with diazotized sulfanilic acid (Diazo reagent) to produce azobilirubin. Caffeine is an accelerator by splitting the unconjugated

Bilirubin protein complex and gives a rapid and complete conversion to azobilirubin. The pink acid azobilirubin is converted to blue azobilirubin by an alkaline tartrate reagent and the absorbance of the blue green solution is measured at (600) nm.

**Reagents:**

- Caffeine- benzoate reagent: (56) g of sodium benzoate and (38) g of caffeine/1 litre
- Sulfanilic acid reagent: (2.5) g of sulfanilic acid
- Sodium nitrite solution: (500) mg of sodium nitrite in (100) distilled water
- Diazo reagent: Mix (4) ml of sulfanilic acid reagent with (0.1) ml of sodium nitrite solution.

***3.10.4.4: Serum AST:***

**Principle of the reaction: -** Aspartate aminotransferase (*AST)* catalyzes the transfer of the amino group from aspartate to oxoglutarate with the formation of glutamate, glutamate and oxaloacetate. The oxaloacetate is converted to malate by malate dehydrogenase; the reaction is monitoring by the change in absorbance at (340) nm continuously as $NADH^+$ is oxidized to $NAD^+$ which is directly proportional to *AST* activity.

**Reagents:**

Reagent (A): Aspartate (362) mmol/l, malate dehydrogenase (460) U/l

Reagent (B): $NADH^+$ (1.9) mmol/l, 2- Oxaloglutarate (75) mmol/l, sodium azide (9.5) g/l.

**Procedure:**

The test was performed as follow:

| | |
|---|---|
| **Temperature** | 37 °c |
| **Sample** | 0.05 ml |
| **Working reagent** | 1.0 ml |

The mixture was thoroughly mixed; in (1) ml cuvette which was inserted immediately into Spectrophotometer *BS* 30, then the activity concentration of *AST* was recorded automatically after (4) minutes.

**Calculation:** AST (U\L) = Δ abs /min x factor

1 M kat/L =60 U/L

***3.10.4.5: .Estimation of Serum ALT:***

***Principle of the reaction: -*** Alanine aminotransferase (*ALT*) catalyzes the transfer of the amino group from alanine to oxoglutarate with the formation of glutamate and pyruvate.

The pyruvate is converted to lactate by *LDH*; the reaction is monitoring by the change in absorbance at (340) nm continuously as $NADH^+$ is oxidized to $NAD^+$ which is directly proportional to *ALT* activity.

**Reagents:**

Reagent (A): Alanine (750) mmol/l, lactate dehydrogenase (1350) U/l

Reagent (B): *NADH* (1.3) mmol/ l, 2- Oxaloglutarate (75) mmol/l, sodium azide (9.5) g/l.

**Procedure:** The test was performed as follow:

| | |
|---|---|
| **Temperature** | 37ºc |
| **Sample** | 0.05 ml |
| **Working reagent** | 1.0 ml |

The mixture was thoroughly mixed; in (1) ml cuvette which was inserted immediately into Spectrophotometer BS 30, then the activity concentration of *ALT* was recorded automatically after (4) minutes.

**Calculation:** ALT (U\L) = ΔAb\min x factor

1 Mkat/L = 60 U/L

### *3.10.4.6: Estimation of Serum ALP:-*

***Principle of the reaction:-***

Alkaline phosphatase *(ALP)* catalyzes in alkaline medium the transfer of the phosphate group from 4-nitropheny-l -phosphate to 2-amino -2- methyle-1-propanol *(AMP),* liberating 4-nitro phenol. The catalytic concentration is determined from the rate of 4-nitrophenol formation, measured at (405) nm.

**Principle:**

Reagent (A): 2- Amino-2- Methyl -1- Propanol (0.4) mol/l, zinc sulfate (1.2) mmol/l, magnesium acetate (2.5) mmol/l.

Reagent (B): 4- Nitrophenylphosphate (60) mmol/l

**Procedure:**

The test was performed as follow:

| | |
|---|---|
| **Working reagent** | 1.0 ml |
| **Sample** | 0.02 ml |

The mixture was thoroughly mixed; in (1) ml cuvette which was inserted immediately into Spectrophotometer BS 30, then the activity concentration of *ALP* was recorded automatically after (4) minutes.

**Calculation:** *ALP* (U\L) = ΔAb\min x factor

Factor = Vt x10

£xI x Vs

The molar absorbance (£) of 4-nitrophenol at (450) nm is (18450), the light path (I) is (1) cm, the total reaction volume (Vt) is 1.02, the sample volume (Vs) is 0.02, and 1 U/L are 0.00166 Mkat/L. the following formulas were deduced for the calculation of the catalytic concentration:

### *3.10.4.7: Estimation of Serum GGT:*

***Principle of the Reaction:***

Gamma-glutamyl transferase catalyzes the *transfer* of the gamma-glutamyl

group gamma-glutamyl-3- carboxy – 4-nitroanalide to glycylglycine, liberating 3- carboxy – 4-nitroaniline. The catalytic concentration is determined from the rate of 3- carboxy – 4-nitroaniline formation.

**Reagents:**

Reagent (A): Glycylglycine 206.3 mmol/l, sodium hydroxide (130) mmol/l.

Reagent (B): Gamma Glutamyl-3- carboxy-4- nitronilide (32.5) mmol/l.

**Procedure:**

Test procedure was performed as follow:

| | |
|---|---|
| **Working reagent** | 1.0 ml |
| **Sample** | 0.1 ml |

The mixture was thoroughly mixed; in 1ml cuvette which was inserted immediately into Spectrophotometer BS 30, then the activity concentration of *GGT* was recorded automatically after 4 minutes.

**Calculation:** GGT (U\L) = ΔAbs/min x factor

Factor = $\frac{Vt \times 10}{£xI \times Vs}$

***3.10.4.8: Blood Urea:***

The urease method for estimation of blood urea was used in this study:

**Principle:**

Urea + $H_2O$ Urease $2NH_4+ + CO_2$

$NH_4+$ + Salicylate + NaClO → Nitroprusside → indophenol.

**Reagents:**

- Reagent $A_1$: Sodium salicylate (62) mmol/l, Sodium nitroprusside (3.4) mmol/l phosphate buffer (20) mmol/l.
- Reagent$A_{2:}$ Urease (500) U/ml
- Reagent B: Sodium hypochloride (7) mmol/l, Sodium hydroxide (150) mmol/l.

### *3.10.4.9: Serum Creatinine:*

Jaffe reaction for estimation of serum creatinine was used in this study.

**Principle of the method used:**

Creatinine reacts with alkaline picrate to produce reddish-orange color the intensity of which at (490) nm is directly proportional to the creatinine concentration.

**Reagents:**

Reagent A: Sodium hydroxide (0.4) mol/l

Reagent B: Picric acid (25) mmol/l

### *3.10.4.10: Estimation of serum Electrolyte:*

The ion-selective electrode (*ISE)* for sodium is often made of a lithium aluminum silicate or other composite silicon dioxide glass compound that selects for ($Na^+$) more readily than ($K^+$) or ($H^+$). The *ISE* for potassium typically contains a selective membrane containing valinomycin.

The valinomycin binds well with the potassium ions. The total plasma carbon dioxide ($tCO_2$) gas cell/electrode contains an acid to convert ($HCO_3^-$) to gas, which diffuses through a silicone membrane and reacts with a bicarbonate/carbonic acid buffer to produce ($H^+$) in proportion to the amount of ($tCO_2$) in the plasma. The ($H^+$) ions are detected by an *ISE* made of silicon dioxide/lithium and calcium oxide glass that selects for ($H^+$) in preference to ($Na^+$) and registers a change in potential versus the silver chloride reference electrode. Chloride can also be measured by an *ISE* of unique composition. A silver chloride membrane solid state electrode measures the activity of ($Cl^-$) and is highly accurate. [146]

**The Principle:**

When the ion comes in contact with the electrode, there is a change in the potential compared to the reference electrode measured as a voltage change, due to the ionic activity. The specimen venous serum or lithium-heparinized plasma.

Electrolytes may also be analyzed in body fluids such as urine, sweat, cerebrospinal fluid, and gastric fluids and are stable if maintained in closed containers and analyzed promptly

### *3.1.: Quality Control:*

Mindray full automated chemistry analyzer was, maintained and calibrated using multicalibrator daily. Biochemistry Control sera level (I) (Normal) and level (II) (Pathological) has been used to verify the performance of the measurement procedure each batch. Results of control sera level (I & II) were within the acceptable range. Biochemistry Control sera level I&II, were from BioSystem S.A. Costa Brava 30, Barcelona (Spain).

# 4. Results

(153) patients were included in this study, (92) of them (60.1) % were male and sixty one (39.9) % were female **(Table 1).** Their mean age was (31.3) years old. Range (12 – 65) years. The control group was composed of (50) individuals thirty one (62) % were male and nineteen (38) % were female. Their mean age was (32.4) years, range (16 – 61) years. (**Fig. 8)** shows the distribution of patients and controls according to age. All patients were ambulant and all of them at that time were living in Shendi locality.

### *4.1: Clinical History:*

The mean duration of symptoms was (4.7) days with range of (1- 17) days. The most common symptoms were fever in (153) patients (100) % and headache in (125) patients (81.7) %, chills occurred in (59) patients (38.5) %, vomiting in (53) patients (34.6) %, diarrhea in (19) patients (12.4) %, urinary symptoms in (44) patients (28.7) % and right hypochondrial tenderness in (2) patients (1.3) %). (**Fig 9)**

### *4.2: Clinical Examination:*

The most common clinical findings were fever in (93) patients (60.7) % with varying degree of severity. Splenomegaly was found in (23) patients (15) %, hepatomegaly in (15) patients (9.8) %. Yellow discolouration of the sclera was seen in (13) patients (8.4) %. Hypotension (BP lower than 90/50) was found in 3 patients (2) %. Two patients showed dehydration due to severe vomiting and diarrhoea. **(Fig. 10)**

### *4.3: Malaria parasites:*

Blood films revealed that 122 (79.7) % of patients had contracted *P.falciparum* malaria of whom71 (46.4) % were male and 51 (33.3) % were

female. *P.vivax* was seen in (31) patients (20.3) % of whom 21 (13.7) % were male and (10) patients (6.6) % were female. **(Table 2)**

### *4.4: Degree of parasitaemia:*

Patients with (+) parasitaemia were 21(13.7) %, those with (++) were (82) (53.6) %, (+++) parasitaemia were 38(24.9) % and (++++) parasitaemia were (12) patients (7.8) %. (**Table 3)** shows degree of parasitaemia and number of patients infected with each plasmodium species. The same table shows that the (++) parasitaemia was the most frequent. (**Table 4**) shows that there is a significant correlation between the duration of symptom and degree of parasitaemia. **(Fig. 11)** shows that the degree of parasitaemia increases with the mean duration of symptoms.

### *4.5: Urine analysis:*

All samples of urine had a *pH* of (5 – 6) except for (3) patients who had a *pH* of (7 – 8). All samples with a *pH* of (7 – 8) had significant pyuria. As shown in fig.6 proteinuria was found in (9) patients (5.9) %, bilirubinuria in (2) patients (1.3) %, increased urobilinogen in (15) patients (9.8) %, glucosuria and ketonuria were not found in all samples. Microscopic examination showed pyuria in (3) patients (2) %, Haematouria in (44) patients (28.8) %, epithelial cells in (5) patients (3.3 %), crystaluria in 6 patients (3.9) % and narrow-bore granular casts in (75) patients (49) %. (**Table 5**) shows the duration of symptoms and urinary findings which was found to be statistically insignificant. ***(P.value 0.13).***

**(Table 6)** shows degree of parasitaemia and percentage of urinary finding which was found to be statistically insignificant in most of findings.

**(Fig. 13)** shows urinary findings in the study group, Proteinuria was found in (9) patients and ranged from (30- 100) mg/dl in (5) patients and from (100- 500) mg/dl in (4) patients. Proteinuria was found to be statistically directly correlated to degree of parasitaemia, **(*P.value 0.01)* (Table 6 &Fig.13)**

but not with the duration of symptoms (table 5). All patients with proteinuria were febrile.

As shown in **(fig. 12)** Bilirubinuria was found in (2) patients (2 crosses). Increased urobilinogen was found in (15) patients, 14(93.3%) of them were infected with *P.falciparum* and one (6.7%) with *p. vivax*. the same patients showed high serum total bilirubin levels, (4.0) mg/dl and (3.2) mg/dl respectively.

Granular casts were seen in (75) patients. All of them were of the narrow – bore type. Statistically they were not correlated to the duration of symptoms, plasmodium species, and degree of parasitaemia or age.

***4.6: chemical analysis:***

All quantitative results of chemical analysis are expressed as mean ± SD. **(Table 7)** shows the means of the different chemical values of both patients and controls.

**(Table 8)** shows the results of the different chemical values of the patients according to the species of the plasmodium. Blood urea was slightly raised in (29) patients (more than 45 mg/dl).the blood urea level in patients was (39.8 ± 15.1) mg/dl while in the control it was (32.5 ± 8.1) mg/dl and this was found to be statistically significant (P.value <0.05) The blood urea value in those infected with *P.falciparum* (39.9 ±14) mg/dl while in those infected with *P.vivax* it was (36.2±15.2) and this was also statistically significant (P.value <0.05).

Creatinine level was increased above normal in (27) patients ranging between (1.5 - 2.4) mg/dl. The Creatinine level in the patients group was (1.3±0.36) mg/dl, while in the control group was (1.0 ±0.24) mg/dl. The difference between these two groups was statistically significant **(P.value <0.05)**. The Creatinine value in patients with *P.falciparum* was (1.4±0.3) mg/dl

while in patients with *P.vivax* was 1.2±0.39 mg/dl. This difference was of no statistical significance (**P.value > 0.05).**

The values of sodium in patients were (130.1±16) mEq/l and that of control were (140.8 ± 2.5) mEq/l and this was statistically significant (P.value < 0.05). The sodium value in patients with *P.falciparum* were (131±6) mEq/l while in patients with *P.viax* were (128 ±15.6). Statistically this was found to be of no significant.

Potassium values in patients were (4.2±1.1) mEq/l and in control were (4.4±0.5) mEq/l. In patients with *P.falciparum* the potassium values were (4.1±1.1) mEq/l, while in patients with *P.vivax* they were (4.2 ±1.0) mEq/l. The difference was not statistically significant. *(P.value > 0.05).*

The total protein values in the patients group were (6.8±0.92) g/dl while in control group they were (6.9±0.33) g/dl. Statistically this was found to be of no significance (P.value > 0.05). Values of serum protein in patients with *P.falciparum* were (6.7±0.7) g/dl while in those with *P.vivax* they were (6.8±0.9) g/dl. The difference was not statistically significant.

Serum albumin in the patients group was (3.2±0.46) g/dl and in the controls group was (3.8±0.31)g/dl. In the patients with *P.falciparum* infection serum albumin was (3.1±0.7) g/dl while in patients infected with *P.vivax* it was (3.2±0.6) g/dl. Statistically this was found to be of no significance (P.value >0.05).

Serum globulins in patients were (3.4±0.91) g/dl while in control group they were (3.1±0.4) g/dl. Serum globulins in patients with *P. falciparum* malaria were (3.3±0.8) g/dl while in *P.vivax* they were (3.4±0.7g/dl). The difference was found to be of no statistical significance (*P.value >0.05).*

Serum total bilirubin level was increased above normal in (69) patients

ranging between (1.1 – 4.0) mg/dl. The total bilirubin level in the patients group was (1.3±0.86) mg/dl; wile in the control group was (0.53 ±0.18) mg/dl. The difference between these two groups was statistically significant *(P.value <0.05)*. The total bilirubin value in patients with *P.falciparum* was (1.3±0.9) mg/dl while in patients with *P.vivax* was (1.4±0.86) mg/dl. This difference was of no statistical significance *(P.value > 0.05).*

The direct bilirubin level in the patients group was (0.55±0.5) mg/dl; while in the control group was (0.27 ±0.11) mg/dl. The difference between these two groups was statistically significant *(P.value <0.05).* The direct bilirubin value in patients with *P.falciparum* was (0.54±0.4) mg/dl while in patients with *P.vivax* was (0.55±0.9) mg/dl. This difference was of no statistical significance (*P.value > 0.05).*

The indirect bilirubin level in the patients group was (0.81±0.39) mg/dl; while in the control group was (0.26 ±0.12) mg/dl. The difference between these two groups was statistically significant (*P.value <0.05*). The indirect bilirubin value in patients with *P.falciparum* was (0.81±0.38) mg/dl while in patients with *P.vivax* was (0.9±0.4) mg/dl. This difference was of no statistical significance (*P.value > 0.05*).

*SGOT* levels in the patient group were (31.8±22.3) IU/L and in the controls group they were (17.4±11.4) IU/L. The difference was found to be statistically significant *(P.value < 0.05). SGOT* levels in patients with *P.falciparum* infection were 30.7±23IU/L while in patients with *P.vivax* infection they were (31.6±22.3) IU/L. (P.value > 0.05). The mean *SGOT* was found to be increased with increased duration of symptoms **(Fig.14).**

*SGPT* in the patients group was (20.3±18.3) IU/L and in the controls group was (12.9±11.1) IU/L *(P.value <0.05). SGPT* in patients infected with *P.falciparum* was (21.3±18) IU/L while in patients infected with *P.vivax* was

(20.1±17.1) IU/L. This was found to be of no statistical significance (*P.value* > 0.05).

Serum alkaline phosphatase *(ALP)* in the patients group was (76±67) IU/L while in the controls group (61.2±29.3) IU/L, the difference was found to be insignificant *(P.value >0.05).* No significant difference was found between *ALP* levels of patients infected with *P.falciparum* (74.9±65IU/L) and patients infected with *P.vivax* (76.8±66IU/L).

Serum *GGT* in patients group was (33.9±21.7) IU/L while in the control group it was (31.1±2.6) IU/L, the difference was found to be insignificant (P.value >0.05). No significant difference was found between serum *GGT* level in patients infected with *P.falciparum* (32.8±22.4IU/L) and those infected with *P.vivax* (33.7±21IU/L) *(P.value >0.05).*

**Table (9)** shows serum biochemical values in patients and control according to sex. Mean serum Creatinine in male was (1.4 ± 0.7) mg/dl and (1.2 ± 0.24) mg/dl in patients and control group respectively, while in female was (1.2± 0.6) mg/dl and (0.8 ± 0.25) mg/dl in patients and control group respectively. The difference was found to be statistically significant. *(P.value 0.01).* There is no significant variation in values of other serum biochemical tests according to sex in patients or control group. (*P.value > 0.05).*

### *4.7: Haematological Parameters:*

**(Table 10 & Fig. 16)** shows the mean of platelets count in patients group was (134000 ±836) C/µL while in controls group was (275±65.7) C/µl. The difference was found to be of statistical significance *(P.value < 0.00).* Platelet count in patients infected with *P.falciparum* was (139±86) C/µL while in patients infected with *P.vivax* was (128±69.2) C/µL. This was found to be statistically significant *(P.value 0.03).* **(Table 11).**

As shown in **(table 10)**, Mean of *MPV* in patients group was (8.5 ±0.99) fl while in controls group was (10.2±0.94) fl. The difference was found to be statistically significant *(P.value < 0.00). MPV* in patients infected with *P.falciparum* was (8.4±0.8) while in patients infected with *P.vivax* was (8.3±0.9). This was found to be of no statistical significance *(P.value 0.23).* **(Table 12)**.

*PDW* in patients group were (16.0 ±0.53) while in controls group were (15.2±0.34). The difference was found to be of statistical significance *(P.value 0.00)*. **(Table 10)**. *PDW* in patients infected with *P.falciparum* was (16.02±0.6) while in patients infected with *P.vivax* was (16.12±0.54). This was found to be of no statistical significance *(P.value 0.34).* **(Table 13)**.

**(Table 14) & (Fig.15)**: shows significant correlation between degree of parasitaemia and thrombocytopaenia, platelet decreased with increased degree of parasitaemia *(P.value <0.005).*

### *4.8: ICT Optimal for malaria:*

**Table (15&16)** shows sensitivity and specificity of *ICT* Optimal method for malaria. Sensitivity was found (95.2) % in mild infection, (98.8) % in moderate infection and (100) % in heavy parasitaemia. Specificity was found (100) % comparing to microscopical method. **(Fig.17)**: shows strong positive correlation between microscopical method and *ICT* (optimal) for malaria diagnosis. (Correlation Coefficient r: 0.99).

**(Table 1): Distribution of patients and controls according to age and sex:**

| **Age group** | **Patients** | | | | **Controls** | | | |
|---|---|---|---|---|---|---|---|---|
| | Male | Female | Total | Percentage | Male | Female | Total | Percentage |
| 10- 20 | 7 | 2 | 9 | 5.9 % | 1 | 0 | 1 | 2 % |
| 21- 30 | 36 | 29 | 65 | 42.5 % | 17 | 12 | 29 | 58% |
| 31 – 40 | 29 | 18 | 47 | 30.9% | 5 | 4 | 9 | 18% |
| 41 – 50 | 14 | 7 | 21 | 13.7 % | 6 | 2 | 8 | 16% |
| 51- 60 | 4 | 4 | 8 | 5.1 % | 1 | 1 | 2 | 4% |
| +60 | 2 | 1 | 3 | 1.9 % | 1 | 0 | 1 | 2% |
| Total | 92 | 61 | 153 | 100% | 31 | 19 | 50 | 100% |

**(Table 2): Distribution of *plasmodium* species according to sex:**

| | **Plasmodium** | | |
|---|---|---|---|
| Sex | *Falciparum* % | *Vivax* % | Total % |
| Male | 71 (46.4%) | 21 (13.7% ) | 92 (60.1 % ) |
| Female | 51 (33.3) | 10 (6.6% ) | 61 (39.9% ) |
| Total | 122 (79.7%) | 31 (20.3% ) | 153 (100%) |

*Chi Square test*

***P.value: 0.03***

**(Table 3): Degree of *parasitaemia* and number of patients infected with each *plasmodium species*:**

| **Degree of *parasitaemia*** | ***P. falciparum*** | ***P. Vivax*** | **Total** | **%** |
|---|---|---|---|---|
| **( + )** | 18 | 3 | 21 | 13.7% |
| **( ++ )** | 64 | 18 | 82 | 53.6% |
| **( +++ )** | 31 | 7 | 38 | 24.9 % |
| **( ++++ )** | 9 | 3 | 12 | 7.8 % |
| Total | 122 | 31 | 153 | 100% |

**(Table 4): duration of symptoms and degree of *parasitaemia:***

| | ***Degree of parasitaemia*** | | | | |
|---|---|---|---|---|---|
| **Duration of symptoms** | + | ++ | +++ | ++++ | Total |
| 0 – 7 days | 17 | 49 | 22 | 10 | 98 |
| 8 – 14 days | 3 | 29 | 14 | 2 | 48 |
| 15 – 21 days | 1 | 4 | 2 | 0 | 7 |
| Total | 21 | 82 | 38 | 12 | 153 |

*Chi square test*

***P. value = 0.02***

**(Table 5): Duration of symptoms and urinary findings:**

| | Duration (in days) | | | |
|---|---|---|---|---|
| Findings | 0 -7days % | 8- 14 days % | 15-21 days % | Total |
| albuminuria | 6 (6.1 % ) | 2 (4.2%) | 1 (14.3% ) | 9 |
| Urobilinogen | 10 (10.2%) | 4 (8.3%) | 1(14.3%) | 15 |
| Bilirubin | 0 (0% ) | 2 (4.2% ) | 0 (0 %) | 2 |
| Haematuria | 38 (38.8 % ) | 4 (8.3%) | 2 (28.6%) | 44 |
| Narrow-bore granular cast | 61(62.2%) | 14 (29.2%) | 0(0%) | 75 |

**Table 6: Degree of *parasitaemia* and urinary finding:**

| | *Degree of parasitaemia* | | | | *P. value* |
|---|---|---|---|---|---|
| Findings | + (%) | ++ ( %) | +++ (%) | ++++ (%) | |
| Proteinuria | 1 (4.8%) | 3 (3.7%) | 4 (10.5%) | 1 (8.3%) | 0.01 |
| ↑urobilinogen | 2 | 9 | 2 | 2 | 0.00 |
| Bilirubin | 0 | 2 | 0 | 0 | 0.08 |
| Haematuria | 8 | 31 | 5 | 0 | 0.13 |
| Narrow-bore granular cast | 8 | 51 | 13 | 3 | 0.11 |

**(Table 7): Serum biochemical values of patients and control:**

| No | Test | Values of patients *Mean ± SD* | Values of Control *Mean+SD* | *P. Value* |
|---|---|---|---|---|
| 1 | Urea (mg/dl) | 39.8 ± 15.1 | 32.5 ± 8.1 | 0.01 |
| 2 | Creatinine (mg/dl) | 1.3 ± 0.36 | 1.0 ± 0.24 | 0.00 |
| 3 | Sodium( meq/l) | 130.1± 16.0 | 140.8± 2.5 | 0.00 |
| 4 | Potassium (meq/l) | 4.4 ± 1.1 | 4.2 ± 0.5 | 0.12 |
| 5 | T.protein (g/dl) | 6.8 ± 0.92 | 6.9 ± 0.33 | 0.58 |
| 6 | Albumin(g/dl) | 3.2 ± 0.46 | 3.8 ± 0.31 | 0.00 |
| 7 | Globulin | 3.4 ± 0.91 | 3.1± 0.40 | 0.01 |
| 8 | T.Bilirubin(mg/dl) | 1.3 ± 0.86 | 0.53 ± 0.18 | 0.00 |
| 9 | D.Bilirubin(mg/dl) | 0.55 ± 0.5 | 0.27 ± 0.11 | 0.00 |
| 10 | Ind.Bilirubin(mg/dl) | 0.81± 0.39 | 0.26 ± 0.12 | 0.00 |
| 11 | SGOT (IU/l ) | 31.8± 22.3 | 17.4 ± 11.4 | 0.00 |
| 12 | SGPT (IU/l ) | 20.3± 18.3 | 12.9±11.1 | 0.008 |
| 13 | ALP (IU/l ) | 76.3 ±67.0 | 61.2 ± 29.3 | 0.12 |
| 14 | GGT (IU/l ) | 33.9 ± 21.7 | 31.1 ± 21.6 | 0.41 |

**(Table 8): Serum biochemical values in patients according to *plasmodium species:***

| No | Test | *P. falciparum* | *P. vivax* | *P. Value* |
|---|---|---|---|---|
| 1 | Urea (mg/dl) | 39.9 ± 14.0 | 36.2 ± 15.2 | 0.00 |
| 2 | Creatinine (mg/dl) | 1.4 ± 0.3 | 1.2 ± 0.39 | 0.01 |
| 3 | Sodium( meq/l) | 131.0 ± 16.0 | 128.0± 15.8 | 0.08 |
| 4 | Potassium (meq/l) | 4.5 ± 1.1 | 4.4 ± 1.0 | 0.15 |
| 5 | T.protein (g/dl) | 6.9 ± 0.7 | 6.8 ± 0.9 | 0.12 |
| 6 | Albumin(g/dl) | 3.1 ± 0.7 | 3.2 ± 0.6 | 0.32 |
| 7 | Globulin | 3.3 ± 0.8 | 3.4 ± 0.7 | 0.09 |
| 8 | T.Bilirubin(mg/dl) | 1.3 ± 0.9 | 1.4 ± 0.86 | 0.16 |
| 9 | D.Bilirubin(mg/dl) | 0.54 ± 0.4 | 0.55 ± 0.9 | 0.20 |
| 10 | Ind.Bilirubin(mg/dl) | 0.81± 0.38 | 0.9± 0.39 | 0.13 |
| 11 | SGOT (IU/l ) | 30.7± 23.0 | 31.6± 22.3 | 0.01 |
| 12 | SGPT (IU/l ) | 21.3± 18.0 | 20.1± 17.1 | 0.21 |
| 13 | ALP (IU/l ) | 74.9 ±65.0 | 76.8 ±66.0 | 0.09 |
| 14 | GGT (IU/l ) | 32.8 ± 22.4 | 33.7 ± 21.0 | 0.12 |

**(Table 9): Serum biochemical values in patients and control according to sex:**

| Test | Controls | | Patients | | *P.value* |
|---|---|---|---|---|---|
| | **Male** | **Female** | **Male** | **Female** | |
| Urea (mg/dl) | 32.5 ± 8.3 | 32.5 ± 8.0 | 38.6±14.1 | 40.1±14.1 | 0.07 |
| Creatinine (mg/dl) | 1.2 ±0.24 | 8.0 ±0.25 | 1.4± 0.7 | 1.2± 0.6 | 0.01 |
| Sodium( meq/l) | 141.0±2.6 | 140.8±2.5 | 131.1±16.0 | 129.1±16.0 | 0.09 |
| Potassium (meq/l) | 4.4 ± 0.4 | 4.4 ± 0.6 | 4.2 ± 1.0 | 4.2 ± 1.3 | 0.16 |
| T.protein (g/dl) | 6.9 ±0.32 | 6.9 ±0.34 | 6.9 ± 0.92 | 6.7 ± 0.92 | 0.22 |
| Albumin(g/dl) | 3.8 ± 0.30 | 3.8 ± 0.32 | 3.3 ± 0.46 | 3.1 ± 0.46 | 0.12 |
| Globulin | 3.1± 0.40 | 3.1± 0.41 | 3.4 ± 0.8 | 3.4 ± 0.92 | 0.19 |
| T.Bilirubin(mg/dl) | 0.53±0.19 | 0.53±0.2 | 1.3 ± 0.84 | 1.3 ± 0.89 | 0.18 |
| D.Bilirubin(mg/dl) | 0.27±0.12 | 0.27±0.11 | 0.54 ± 0.7 | 0.56 ± 0.3 | 0.23 |
| Ind.Bilirubin(mg/dl) | 0.26±0.12 | 0.26±0.11 | 0.82± 0.38 | 0.80± 0.4 | 0.09 |
| SGOT (IU/l ) | 18.6±11.4 | 16.2±11.3 | 32.6± 21.3 | 30.9± 23.3 | 0.17 |
| SGPT (IU/l ) | 13.1±10.0 | 12.9±11.1 | 22.1± 18.0 | 19.9± 18.6 | 0.26 |
| ALP (IU/l ) | 61.2±29.3 | 61.2±29.3 | 76.8 ±66.9 | 76.1 ±67.1 | 0.09 |
| GGT (IU/l ) | 31.2±21.6 | 31.0±21.6 | 33.7 ± 21.6 | 33.8 ± 21.0 | 0.21 |

**(Table 10): Mean platelet count, *MPV* and *PDW* in patients & Controls:**

| No | Test | Values of patients *Mean ± SD* | Values of Control *Mean+SD* | *P. Value* |
|---|---|---|---|---|
| 1 | Platelet count | 134.000 ± 83.6 | 275 ± 65.7 | 0.000 |
| 2 | Mean platelet volume | 8.5± 0.99 | 10.2 ± 0.94 | 0.000 |
| 3 | Platelet Distribution width | 16.0 ± 0.53 | 15.2 ± 0.34 | 0.000 |

**(Table11): Mean of platelet count in patients according to type of *plasmodium species*:**

| No | *Plasmodium Species* | Platelet count/µl | *P.value* |
|---|---|---|---|
| 1 | *P. Falciparum* | 139.400± 86 | 0.03 |
| 2 | *P. Vivax* | 128.900± 69.2 | |

**(Table 12): Mean of *MPV* in patients according to type of *plasmodium species:***

| No | Plasmodium Species | MPV | *P.value* |
|---|---|---|---|
| 1 | *P. Falciparum* | 8.4±0.8 | 0.23 |
| 2 | *P. Vivax* | 8.3± 0.91 | |

**(Table 13): Mean of *PDW* in patients according to type of *plasmodium species:***

| No | *Plasmodium Species* | *PDW* | *P.value* |
|---|---|---|---|
| 1 | *P. Falciparum* | 16.02± 0.6 | 0.34 |
| 2 | *P. Vivax* | 16.12± 0.54 | |

**(Table 14): Thrombocytopaenia according to degree of parasitaemia:**

| **Parasitaemia** | **Frequency** | **Percentage** | **Mean of platelet count/µl** | ***P.value*** |
|---|---|---|---|---|
| + | 21 | 13.7% | 143.000 | 0.007 |
| ++ | 82 | 53.6% | 136.000 | |
| +++ | 38 | 24.9 % | 133.000 | |
| ++++ | 12 | 7.8 % | 117.000 | |

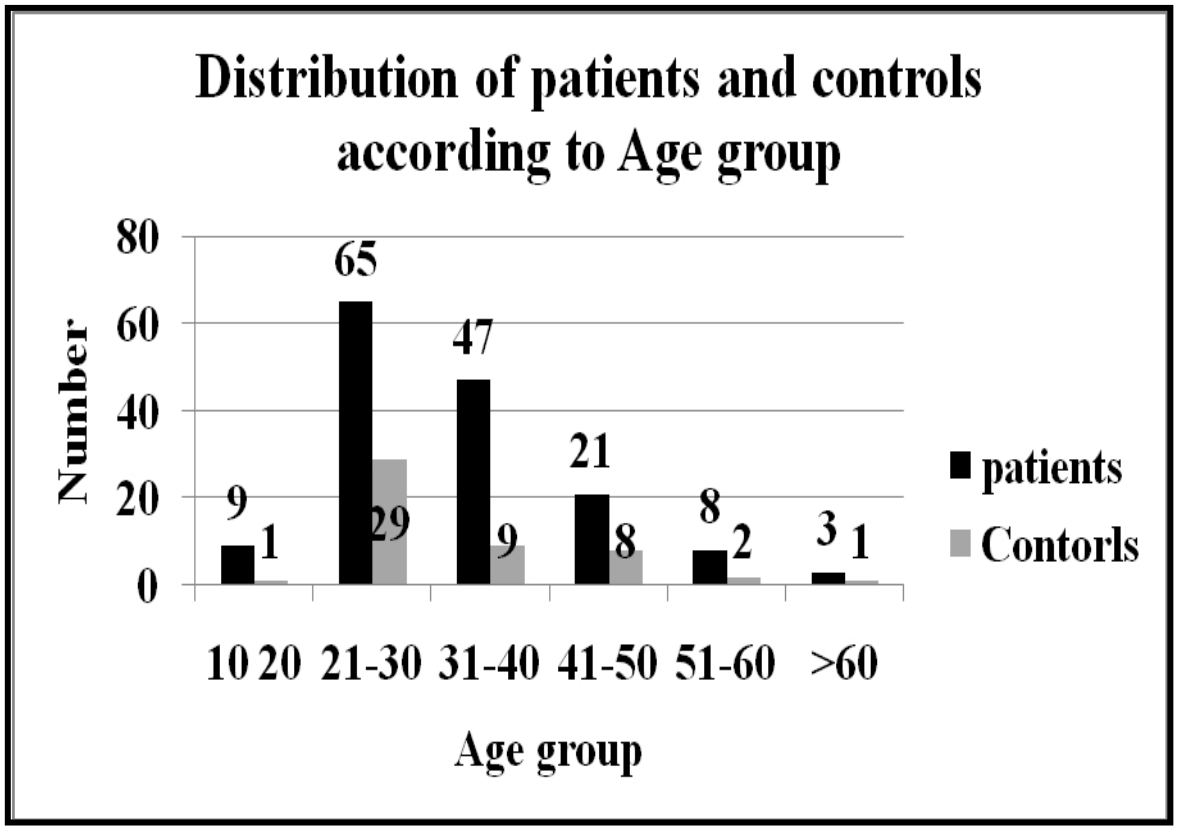
**(Table 15): Sensitivity of *ICT* (optimal) for malaria diagnosis according to degree of parasitaemia:**

| Parasitaemia | Microscopical Method | ICT optimal | Sensitivity % |
|---|---|---|---|
| + | 21 | 20<br>(TP=20, FN=1) | 95.2 % |
| ++ | 82 | 81<br>(TP=81, FN=1) | 98.8 % |
| +++ | 38 | 38<br>(TP=38, FN=0) | 100% |
| ++++ | 12 | 12<br>(TP=12, FN=0) | 100% |

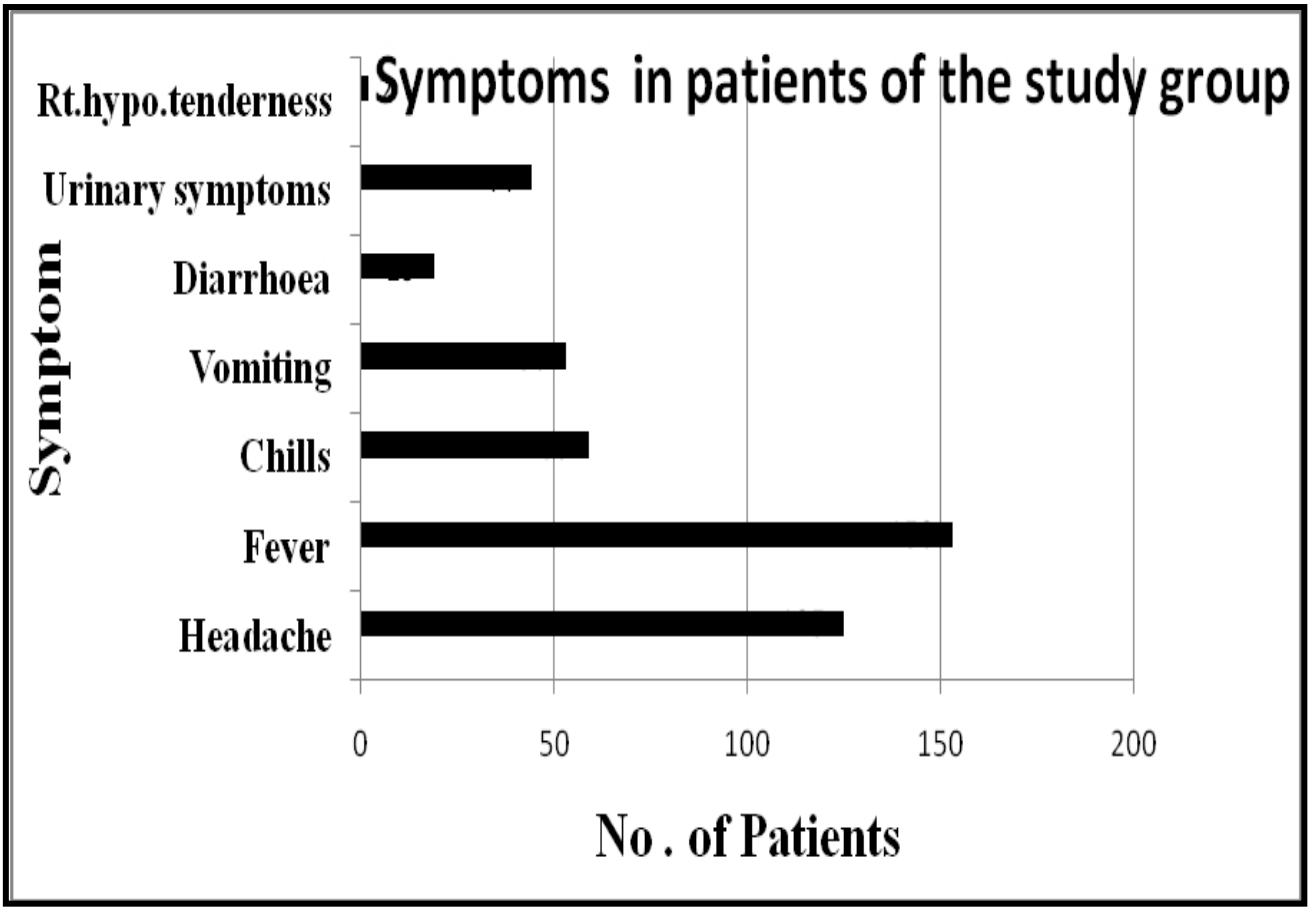
**(Table 16): Specificity of *ICT (*optimal) for malaria diagnosis:**

| **Number of tested individuals (Controls)** | **Type of Method (Test)** | | **Specificity** |
|---|---|---|---|
| | **Microscopical** | **ICT Optimal** | |
| 50 | All 50 results were negative (TN) | All 50 results were negative (TN= 50, FP= 0) | 100 % |

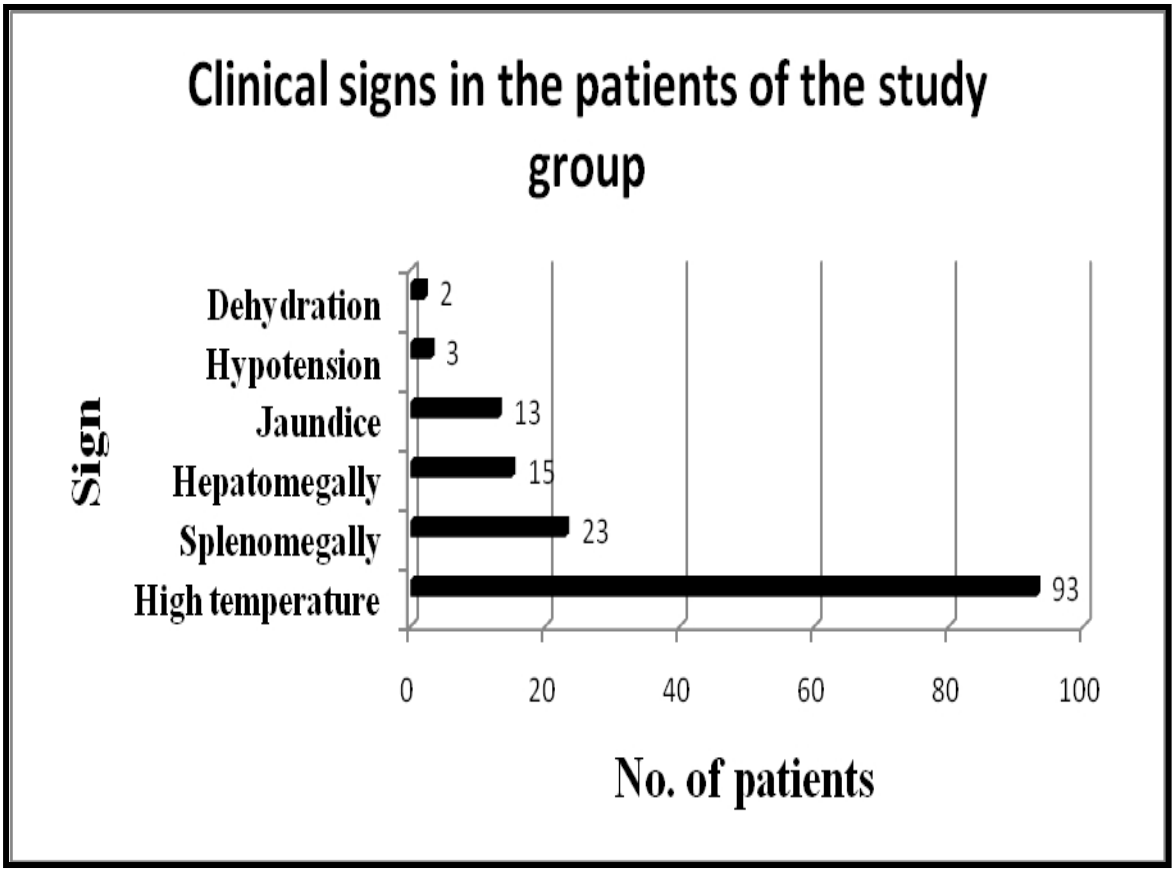
**(Fig.8): Distribution of patients and controls according to age group:**



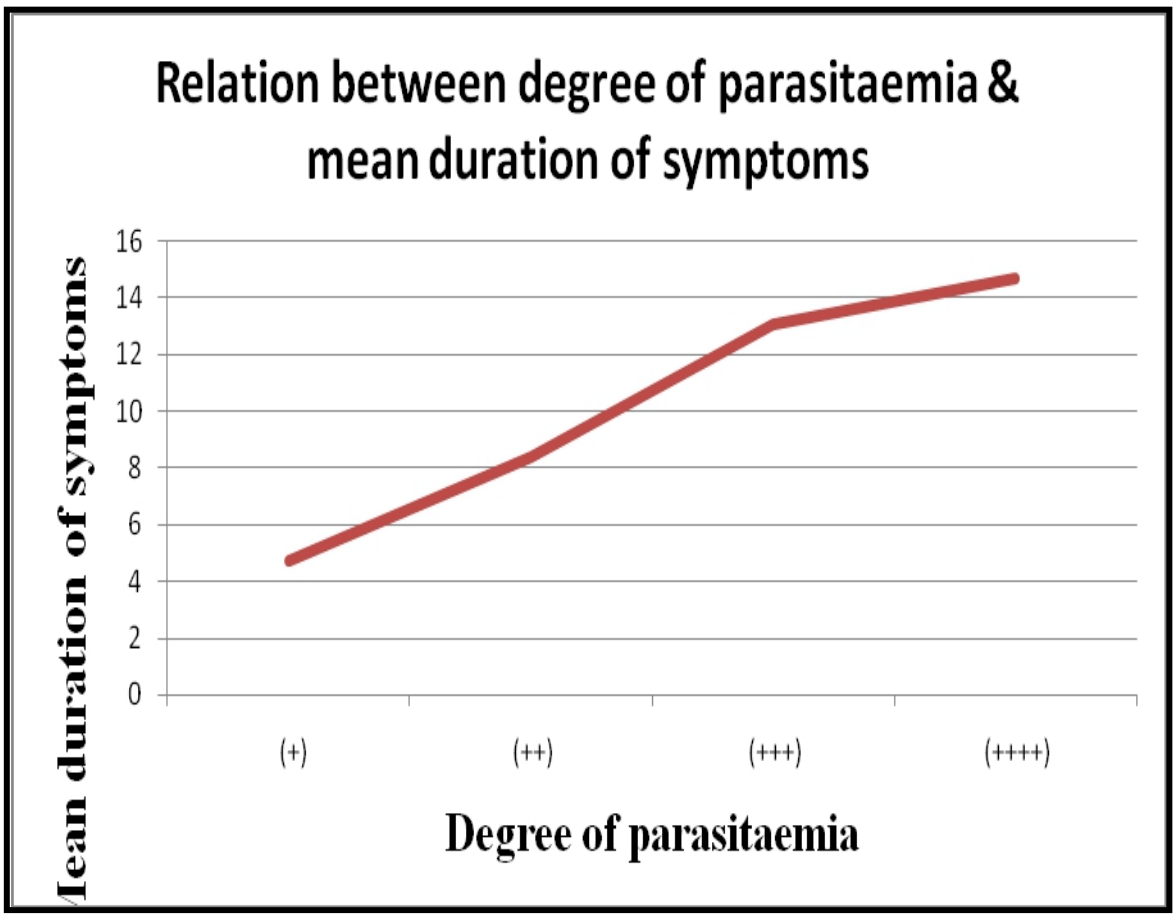
**(Fig.9): Symptoms in patients of the study group:**



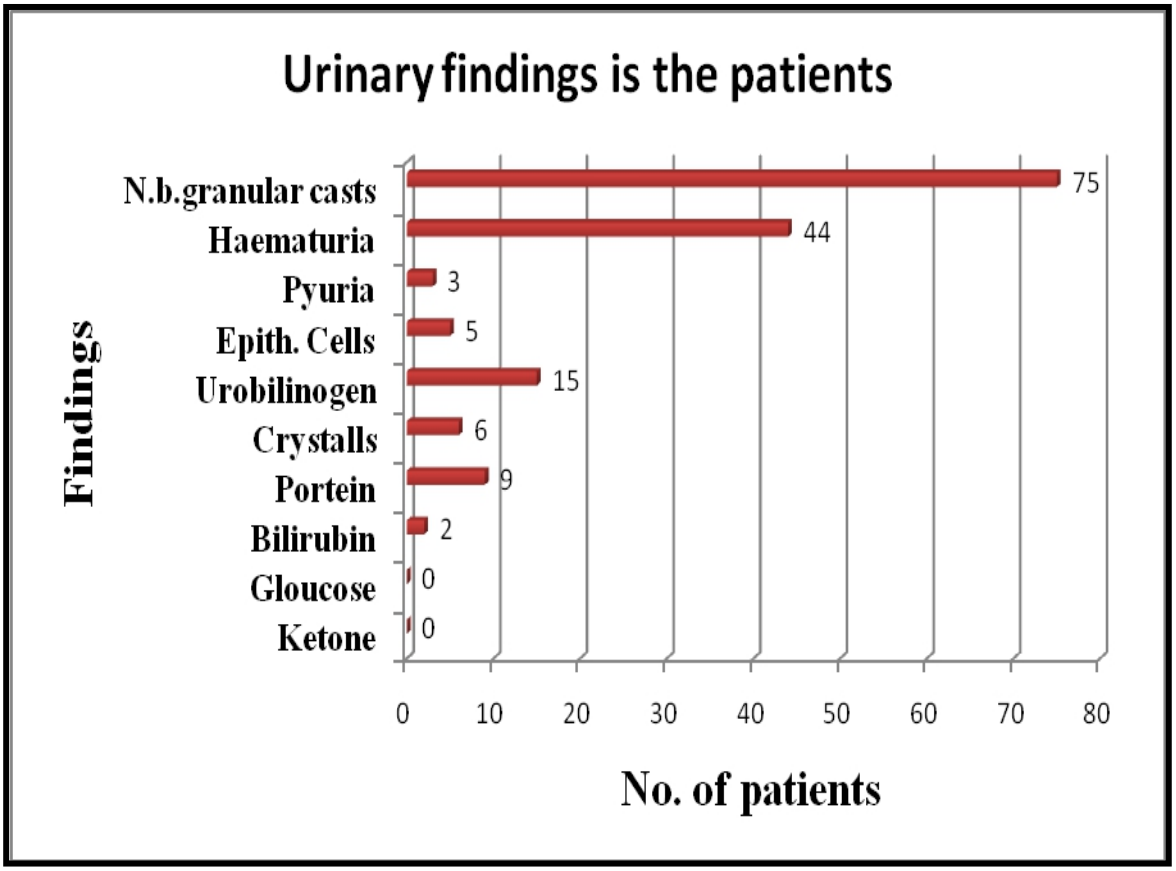
**(Fig.10): Clinical signs in the patients of the study group:**



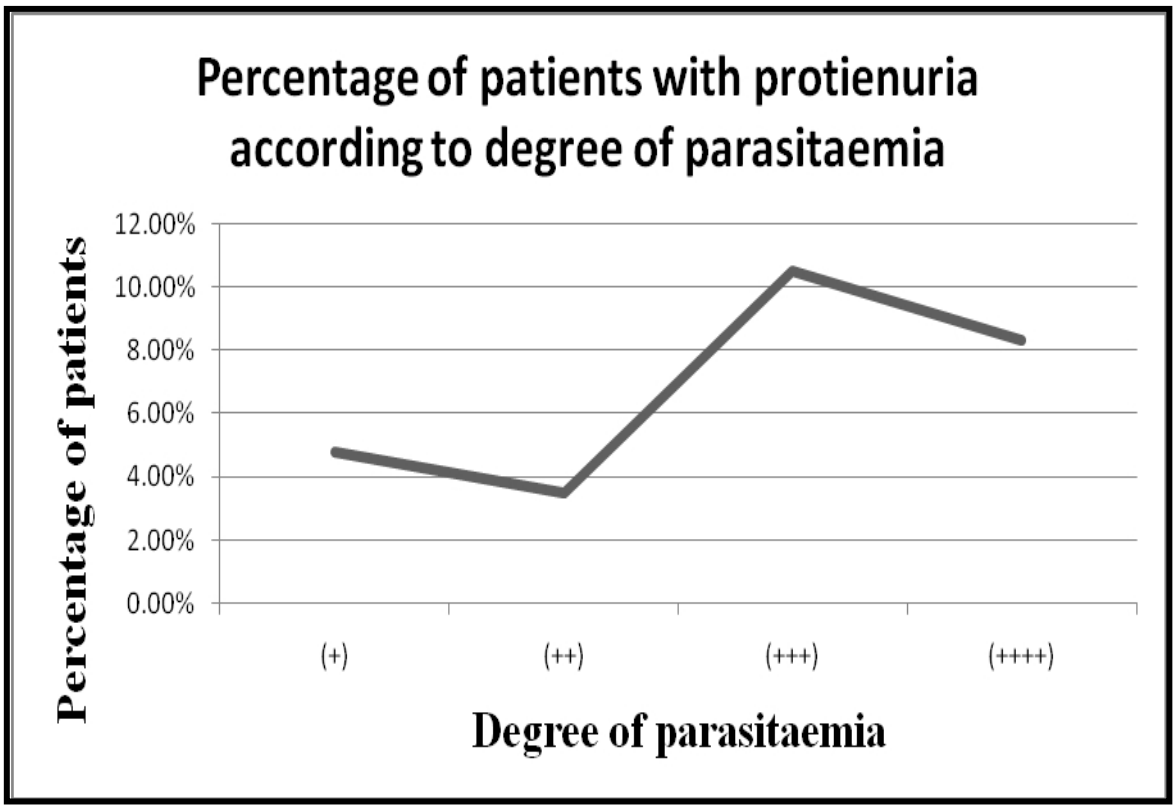
**(Fig.11): Relation between degree of parasitaemia & duration of symptoms:**



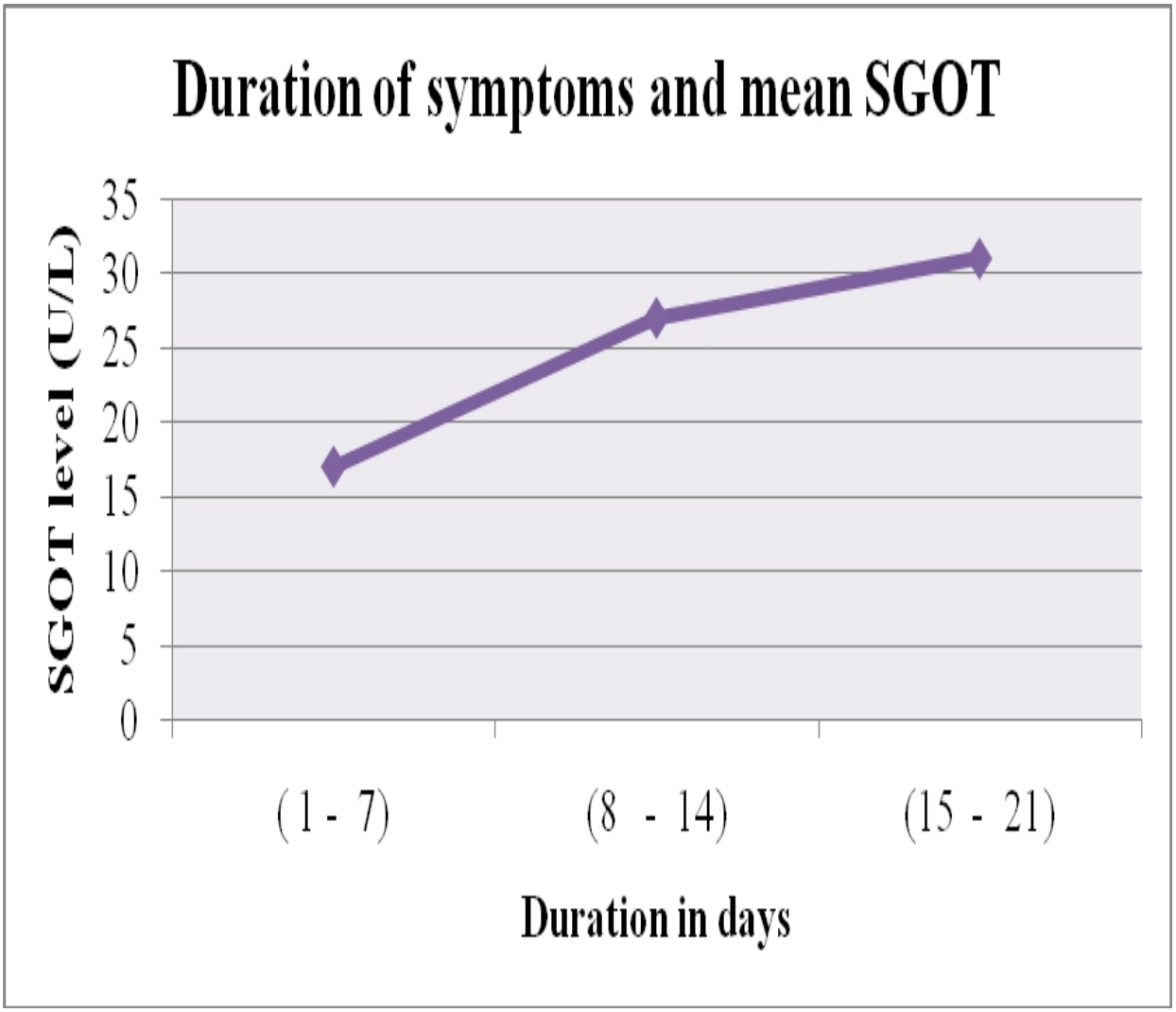
**(Fig: 12): Urinary findings in the patients**



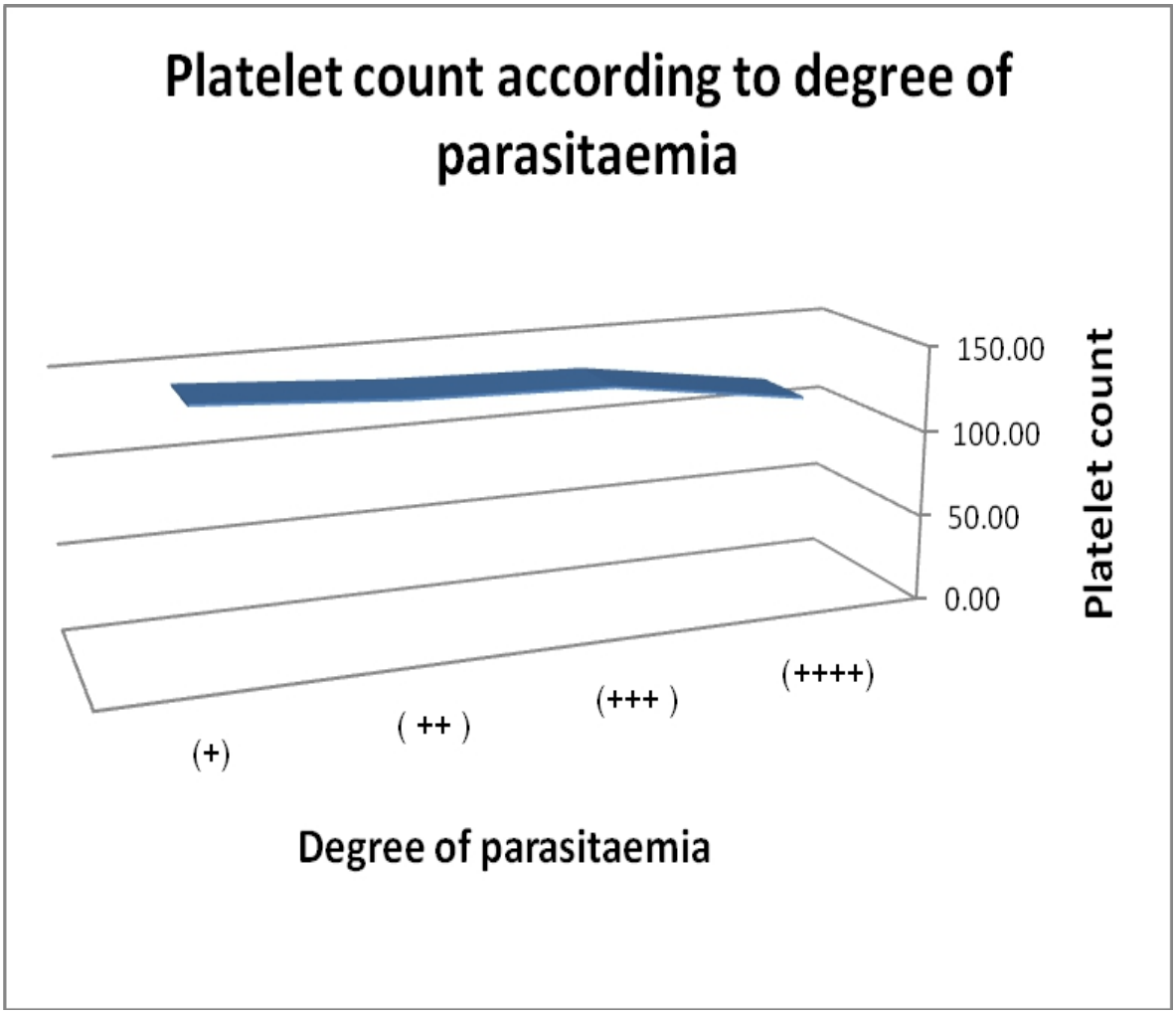
**(Fig.13): Percentage of patients with proteinuria according to degree of *parasitaemia*:**



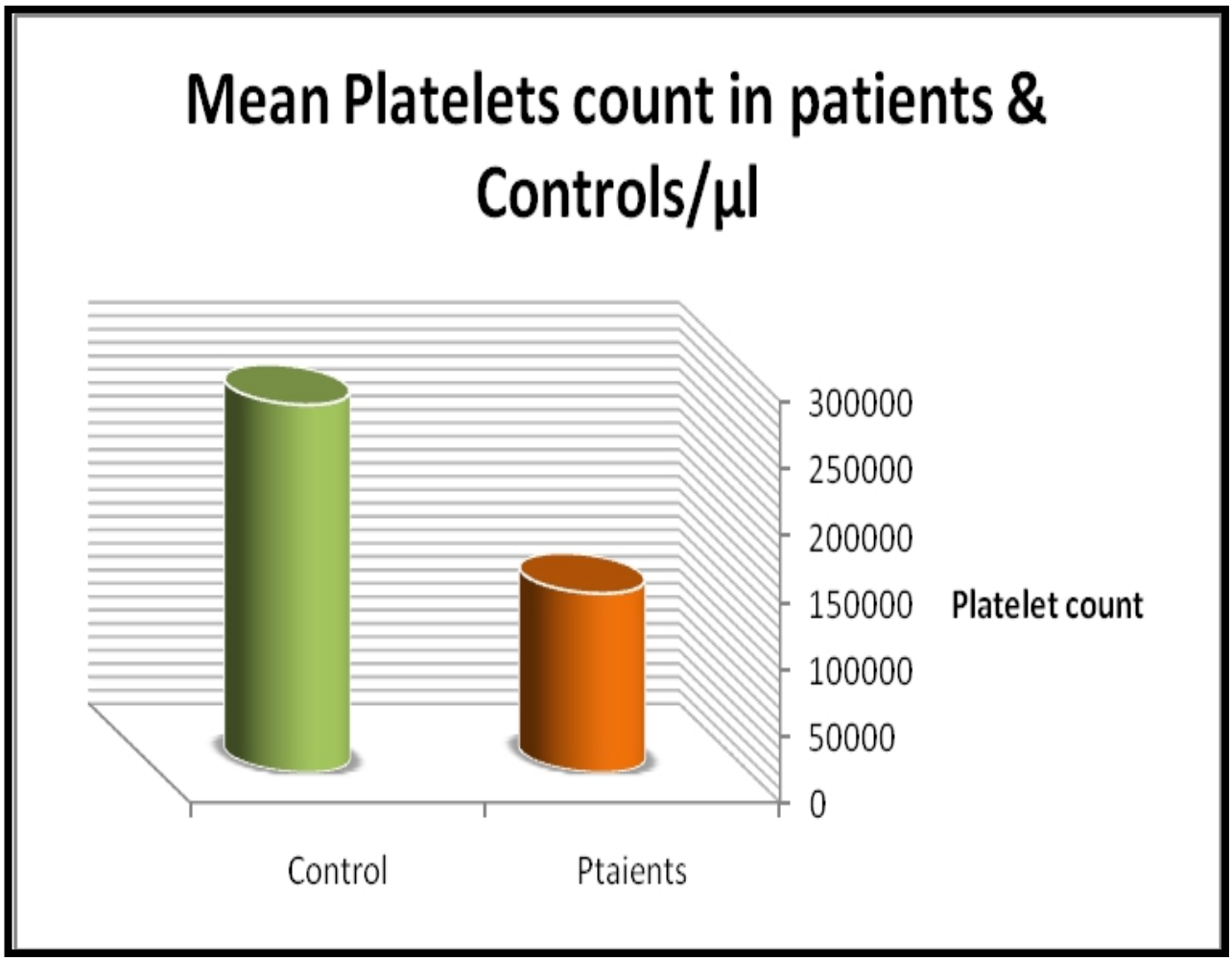
**(Fig.14): Duration of symptoms and mean *SGOT*:**



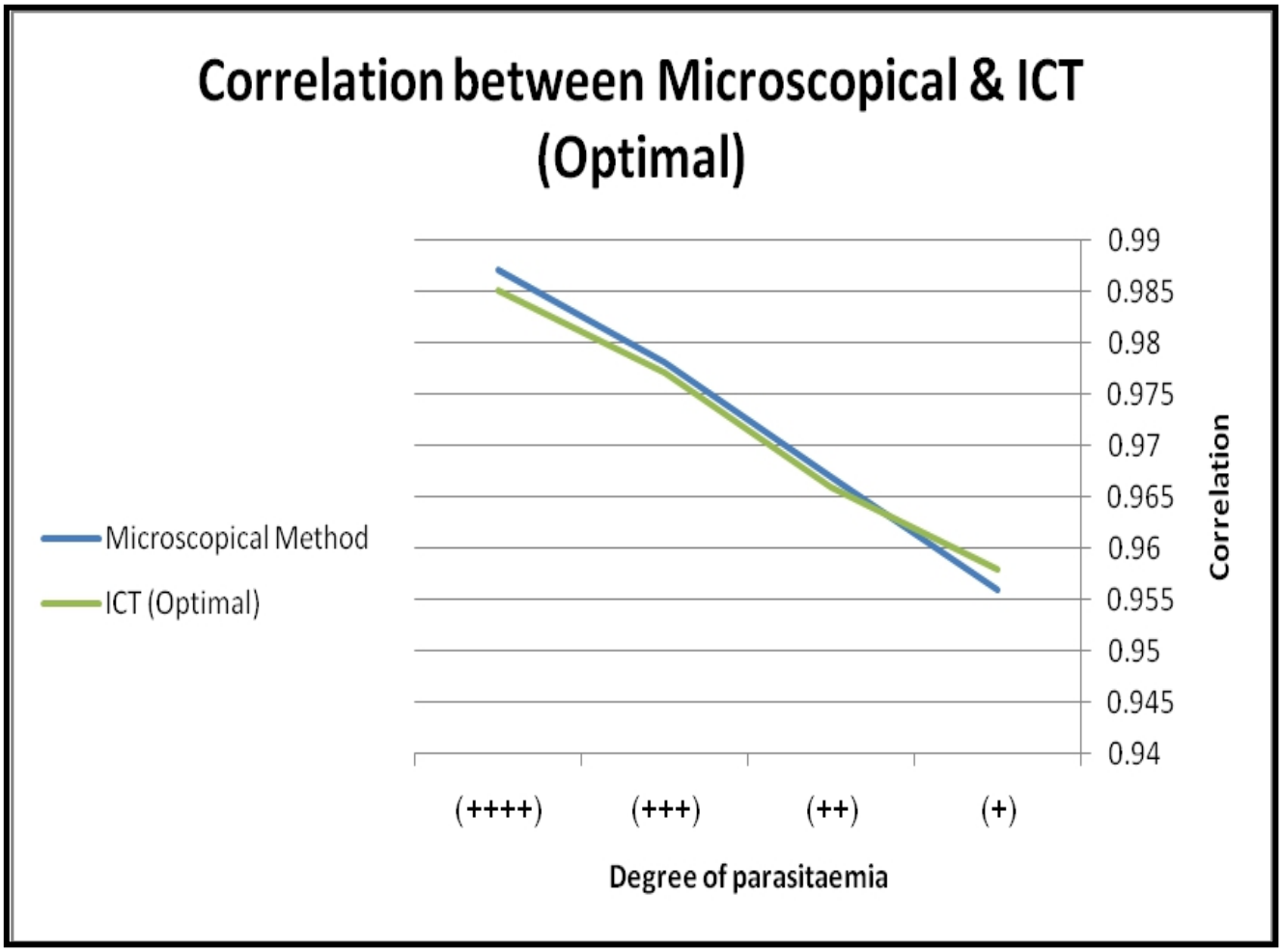
**(Fig.15): platelet count according to degree of *parasitaemia*:**



**(Fig. 16): *Mean platelet count* in patients and control/µl of blood:**



**(Fig.17): Correlation between microscopical method and *ICT* (Optimal):**



## 5.1: Discussion

***General Consideration:***

This study was conducted in Shendi locality, River Nile state which is an area of hypo endemic malaria. The majority of samples were collected from the laboratory department in different hospitals (*Shendi hospital, Elmek Nimer University hospital, military hospital, Elmiseaktab hospital, Elnourab hospital*) and different private clinics and laboratories.

Most of the Sudanese consider any febrile illness or generalized aches and fatigability as malaria and due to the high incidence of malaria infection thus a great numbers of them use anti malarial treatment without examining their blood for the presence of the parasite.

***Urine examination:***

Proteinuria was found in only (9) patients ranging from (30– 500) mg/dl. It was statistically correlated to fever, but not to duration of symptoms or plasmodium species. Fever is a known contributing factor in proteinuria, a rise in urine protein (150 – 1500) mg/day was found in (12) patients out of (27) patients with *P.falciparum* infection [(147)].

Granular casts were found in (75) patients in this study. They were of the narrow-bore type and their number differed from one sample to another but they were usually of low number. There was no significant statistical correlation between these narrow – bore granular cast and age, sex, parasitaemia or plasmodium species. This type of granular cast was observed by *Dr. M.D.Mustafa* and *Dr. G.M.Elimari* in (111) patients out of (213) patients with malaria. [(130)]

In this study increased urobilinogen was found in (15) patients while bilirubinuria was found in two patients. The fifteen patients mentioned above had elevated unconjugated hyperbilirubinaemia, range of serum bilirubin from (1.1 – 4.0) mg/dl and the range of unconjugated bilirubin from (0.8 – 3.8) mg/dl.

It has been suggested that destruction of red cells may result from the operation of different pathophysiological mechanism:

1. The mechanical destruction of red cells by the malaria trophozoite forms growing inside them [149]. The trophozoite divides repeatedly forming the merozoites and ultimately causing rupture of the red cells release of the merozoites into the circulation in order to attack further red blood cells [149]. The high degree of haemolysis resulting from this mechanism had been attributed to the ability of *P.falciparum* to enter red cells of all ages with special predilection for young red cells [149]. In addition the membranes of infected erythrocytes showed major alterations in their components which might be responsible for the increased osmotic fragility, membrane fluidity and passive permeability [150].

2. A role for immunity in red blood cells destruction in *P.falciparum* malaria was postulated. Opsonization and erythrophagocytosis following red blood cell sensitization by either the adherence of immune complexes or by *IgG* and or complement components [151]. This may occur in the absence of parasitaemia. Autoimmune mechanisms were also suggested for the red blood cell destruction in malaria [152], in which *IgM* antibodies are formed against normal erythrocytes resulting in their increased destruction. It was suggested that these *IgM* antibodies occur in individuals in endemic areas exposed to malaria since birth [152].

3. Another suggested mechanism is that parasitized erythrocytes may undergo membrane changes and become less deformable, thus resulting in their premature clearance by the spleen macrophage [153].

4. Biochemical and morphological abnormalities in both parasitized and non parasitized erythrocytes had been observed. The increased osmotic fragility of erythrocytes during malaria infection had been attributed to altered cation permeability across the erythrocyte membrane, which results in abnormal sodium and potassium concentrations inside the erythrocytes [39, 40]. The aetiology of destruction of the mature non parasitized erythrocytes is attributed

to many suggestions. One of the suggestions is that soluble malarial antigen – antibody complexes may become adsorbed to the erythrocytic membrane, fix complement and cause immune haemolysis with spherocytes which are frequently seen in immune haemolytic anaemia[s (154)].

In our study bilirubinuria was detected in (2 ) of the patients. Serum total bilirubin was (4.0) mg/dl in the first patient and (3.2) mg/dl in the second. Conjugated bilirubin of the (2) patients was (2.1) mg/dl and (2.7) mg/dl respectively. In the first patient it was clear that he also had a raised level of unconjugated bilirubin and his urine showed increased urobilinogen. The serum transaminase in both patients were elevated, *SGOT* was (118) IU/L and (97) IU/L respectively.

Conjugated hyperbilirubinaemia was observed in patients infected with malaria, Chawla et al found that (65%) of their (32) malarious patients had conjugated hyperbilirubinaemia, (21.5%) had unconjugated hyperbilirubinaemia and (21.8%) had a mixed pattern. Transaminases were elevated in (21.8%). Histologically they found reticuloendothelial cell hyperplasia, pigmentation in kupffer cells, fatty changes, sinusoidal and portal mononuclear infiltration and cholestasis [(155)].

Mishra and his colleagues [(156)] found that in (33) jaundiced patients out of (165) hospitalized malarious patients, (22) had unconjugated hyperbilirubinaemia while (11) had conjugated hyperbilirubinaemia. They concluded that acute malarial hepatitis is a relatively uncommon complication in *P.falciparum* malaria. The two patients with conjugated hyperbilirubinaemia in this study need further investigations to reach a cause for the increased direct bilirubin and to confirm whether this elevated conjugated hyperbilirubinaemia was due to malarial hepatitis or to congenital conjugated hyperbilirubinaemia or other possible causes.

In this study there is a significant variation in mean of *SGOT* & *SGPT* levels between patients and controls; also there is a significant correlation

between the level of *SGOT* and duration of symptoms. This can be explained by the fact that parasitized red blood cells are exposed to mechanical destruction by the malaria trophozoite forms growing inside them, and as mentioned before, degree of parasitaemia in this study increased with the increased mean duration of symptoms, so the longer duration of symptoms is expected to lead to increased haemolysis and hence cause a rise in *SGOT*.

Other studies differ in their opinion concerning levels of *SGOT*. Some found no change in *SGOT* level in malaria infection of man but in animals they reported elevations in *SGOT* [157] which was explained by concurrent haemolysis. SGOT in man was noticed to be elevated in malaria in (60) % of the patients and associated with either liver tenderness, gastrointestinal symptoms or both [158].

As shown in **(table 7)** there is a significant difference between means of *SGPT* in patients and controls.

The results obtained are agreed with other studies which showed variable degree of *SGPT* elevation. Serum *SGPT* was increased in studies done by van *Fuhrman* and *Peltzer* [159]. *Sauden et al* reported a significant increase over values in uninfected controls in *SGPT* levels [157]. *Deller* and his colleagues also reported *SGPT* elevations in (60) % of their patients [160].

*Chawla* and his colleagues found that *SGPT* was increased mildly in (3) % of their patient [155]. *Patwari* in studying hepatic function in childhood *P.vivax* malaria found markedly raised levels of *SGPT* in a large proportion of patients with hepatomegaly [161].

In a study conducted in 2012 in Sudan, *Elnoman E. et al* they report that Patients with malaria, had increased level of *AST*, *ALT*, total bilirubin and indirect bilirubin. [175]

In our study there is statistically no significant difference between mean serum alkaline phosphatase *(ALP)* level in both patient and control groups. This agrees with the studies done by Sauden et al. [157] and Deller et al [158].

*Mishra* and his colleagues reported an increase in *ALP* in (11) patients [156], while *Patwari* reported an increase in (50) % of the patients with hepatomegaly and in (38) % of the patients without enlarged liver suggesting an obstructive component in the disease process [161].

In our study no significant difference was found between mean serum Gamma Glutamyl Transferase (*GGT*) of both patient and control groups. All previous studies consulted except one, did not report on the serum *GGT* level in malaria infection. *Mishra* and his colleagues were the only investigators who reported an increase in serum *GGT* level in (3) out of (165) patients infected with malaria [156].

In this a study, as shown in **(table 7)** there is no significant difference between mean total protein in both patient and control group. Albumin was found slightly decreased in patient group while plasma globulin was increased in patient with malaria. Hypoalbuminaemia can be dillutional, due to redistribution of albumin in the body fluids due to prolonged bed rest or due to decreased synthesis of albumin as in chronic liver disease or malnutrition. The normal value of plasma total protein in our patient although there was slightly decreased of albumin; but compensated with increase of plasma globulin.

As shown in **(table 7&8)** mean serum urea and Creatinine levels in patients group were higher than in the controls group. The serum urea and creatinine level in patients with *P.falciparum* infection was higher than patients with *P.vivax* infection.

Our study shows that malaria in general and especially *P. falciparum* malaria may cause elevation in blood urea level. These results agree with the findings of the majority of the studies reported and consulted. *Benyajati* and his

colleagues found elevated blood urea and Creatinine level in about (25) % of their patients. [164].

*Sitiprija* et al observed moderate azotaemia in (14) % of their cases with *BUN* ranging between (38 – 80) mg/dl and serum Creatinine level from (1.0 – 2.5) mg/dl. [165]. *Miller* and his colleagues reported little evidence of azotaemia. [166] *Sitiprija* also reported transient renal failure in (3) malarial patients with raised plasma urea levels (82 – 194 mg/dl) and Creatinine levels of (2.0 – 3.6) mg/dl which returned to within normal limits by the fourth or fifth day of admission after anti malarial treatment. [167].

*Stone et al*. studied (42) patients and he found oliguria in (36) patients. He also found sever azotaemia with some *BUN* level of over (200) mg/dl and Creatinine levels up to (15) mg/dl or more. [168]. Reid and his colleagues investigated a British seaman who contracted *P.falciparum* infection and was found to have a blood urea of (255) mg/dl and serum Creatinine of (7.5) mg/dl. Following anti malarial treatment and dialysis he improved. [169].

Ahmed and his colleagues studied renal function in (75) malarious patients. They found significant reduction in endogenous Creatinine clearance. [170]. *Sidhn* and his colleagues reported that; pernicious syndromes: (anaemia, thrombocytopaenia, hyponatraemia and renal failure) occurred more often in P.falciparum malaria.

*Ekeanyanwu* and his colleagues report that the levels of serum urea, serum creatinine and protein in urine in the infected volunteers had higher mean concentration and these differences were statistically significant at $p<0.05$ when compared with the control counterparts. [171]

In this study there is a significant variation was found between the mean serum sodium in patients and controls. No significant difference was found in the mean serum sodium level of patients infected with *P.falciparum* and patients infected with *P.vivax*.

Our result is agreed with *Miller* and his colleagues who noticed mild hyponatraemia in all their patients except two who were seen during the first week of the malaria attack. This hyponatraemia was attributed by them to a mixture of salt depletion and water retention. In some of their patients they suggested inappropriate secretion of anti diuretic hormone *(SIADH)* as the cause of hyponatraemia. [166]. Hyponatraemia, considered as one criterion of the pernicious syndrome was found more often in *P.falciparum* infection.([172].

In this study the mean serum potassium in both patient and control groups was found to be of no statistically significant difference as shown in **(table 7).**
No significant variation was found in mean serum potassium between the two groups of patients infected with either *species of plasmodium.* However there are (15) patients had a slightly increase in serum potassium range from (5.1) to (6.2) meq/l.

Our findings are in line with *Stone et al* when studying (24) malarial patients found hyperkalaemia in only (5) patients. [168]. but contrast with hypokalaemia reported in (27.3) % of Indians admitted for severe malaria infected with evidence of severe renal derangement which reported by *Ebele J et al*. and also differ from hyperkalaemia in malaria patient reported by *Etim O.E etal.* [173]

The decrease in concentration of sodium may be due to losses in sweat and urine. Losses in urine may serve to compensate for increased blood urea concentrations found in *falciparum malaria infection*, in order to maintain constant body osmolality. Reduction of sodium concentration by increased urea, and other somatically active solutes have been considered appropriate if constant osmotic gradient between cells and the extra-cellular fluid compartment is maintained.
Furthermore, increased urea and creatinine level in these patients suggests that the ureamia observed in this study is largely prerenal and may be due to reduced renal blood flow and glomerular filtration rather than organic renal involvement.

As shown in **(table 10)** there is a significant variation in mean of platelet count, mean platelet volume and platelet distribution width. The results revealed a high frequency of thrombocytopenia and changes in *MPV* and *PDW*. In this study, platelet counts were significantly reduced in malarial infected subjects. Thrombocytopaenia occurred in (67.5) % of malarial cases in comparison to study done in Pakistan which had high percent (85.5) %. Effect of *P.falciparum* on platelet count showed thrombocytopaenia with mean of platelet count of (139,400/µl), *P.vivax* were thrombocytopaenia with mean of platelet count of (128,900/µl) in comparison to study achieved in India showed the mean of platelet count in *P.falciparum* infection was (100,900/µl) and in *P.vivax* was (115,390/µl) and *P.malairae* were thrombocytopenia with mean of platelet count of (103,000/µl). [176].The trend of decreasing platelet count with increasing levels of parasitaemia observed in this study has been previously noted by (Eze Evelyn M et al. 2012). [177]

Many recent studies have found thrombocytopaenia associated with *P. vivax*, *Sheikh MA et al* report different degree of thrombocytopaenia in (171) patients out of (200) malaria patients included in their study.[178]

In general, the underlying mechanisms of thrombocytopenia in malaria are peripheral destruction, excessive sequestration of platelets in spleen, and excessive use of platelets associated with the disseminated intravascular coagulation phenomenon In addition to the reduction in the number of platelets, platelet function is also compromised in these patients; this is generally evidenced by changes in the volume and other features of platelet cells. [179]

As shown in **(table 15, 16)** the sensitivity of optimal test for rabid diagnosis of malaria is (95.2) % for mild parasitaemia, (98.8) % for moderate parasitaemia and (100) % for heavy parasitaemia. *ICT optimal* reveals (100) % specificity for malaria diagnosis.

Carol J et al report that sensitivity of *optimal* (Flow Inc., Portland), to detect *P. falciparum* malaria were (99) % and specificity of (100) % for malaria

diagnosis when compared to those obtained by reading (100) fields of traditional Giemsa-stained thick-smear blood films for the detection of *P. vivax* and *P.falciparum* malaria. [144] This results is approximately agreement with the findings in our study.

## 5.2: Conclusion

From this study it seems that liver function in acute malaria is not significantly disturbed. In the few cases in which an abnormality of liver function tests was noted, further study is needed to delineate the cause of these abnormalities. All patients in this study were ambulant and a study of severely ill hospitalized patients is needed for a meaningful comparison with the available literature. Renal function in this study was shown to be slightly disturbed as evidenced by the slightly significant difference in serum urea, Creatinine and sodium of patients compared to controls.

P. falciparum seems to affect renal function more than *P. vivax*. Platelet count in this study was shown to be significantly reduced in patients compared to controls, and thrombocytopaenia is directly proportional to degree of parasitaemia. *P. vivax* seems to altered platelet count more than *p. falciparum*.

## 5.3: Recommendations

1. All patients with malaria should be subjected to routine kidney and liver function tests to rule out renal and/or liver impairment, especially in all cases of severe malaria as early diagnosis will significantly reduce mortality rate.
2. Further studies of liver and renal function in hospitalized patients with malaria.
3. Study of the effects of various antimalarial treatments, which was not included in this study, on liver and renal function is needed.
4. Constant re-evaluation of the incidence and prevalence is done, preferably a country wide study in order to establish the true picture of the incidence and prevalence in Sudan.
5. Further studies on renal and liver function tests in different types of *plasmodium species*.
6. Altered platelet indices should be analysed as potential markers for the severity of malaria caused by *P.falciparum & P. vivax*.
7. *ICT* for malaria antigen should be used as well as a microscopical method especially by a newly laboratory technologist in absence of senior laboratory supervisor.
8. Well training of laboratory technologist on microscopical examination of malaria.

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بسم الله الرحمن الرحيم

**University of Shendi**

College of Graduate studies

# Renal, Liver Function Tests and some Haematological Prameters in Malaria Patients in Shendi Locality, River Nile State, Sudan.

## *Questionnaire*

## *A-Personal data:*

Patient Name:…… Date:…….. Age:

1-20 years ☐ 21–40 years ☐ 41-60 years ☐ Over 60 years ☐

Gender: Male ☐ Female ☐

Residence: .....

Occupation: .....

## *B- Clinical Presentation:*

1. Duration (------) day. 2. Fever: Yes ☐ O ☐

3. Headache: Yes ☐ No ☐ 4. Vomiting Yes ☐ ☐

5. Diarrohea: Yes ☐ NO ☐

6. RT. Hypoch. Pain: Yes ☐ NO ☐

7. Loin pain: Yes ☐ NO ☐ 8. Dysuria (Yes…) (NO...)

9. Others……

10. Urine volume: Normal ☐ Increased ☐ Decreased ☐

## *C- Past history:*

1. Hypertension: Yes ☐ No ☐ . U.T. Disease Yes ☐ o ☐

3. Liver Disease: Yes ☐ No ☐ .D.M: Yes ☐ No ☐

5. Others……

## *D- Physical Examination:*

1. Anaemia: Yes ☐ No ☐ 2. Jaundice: Yes ☐ No ☐

3. Dehydration: Yes ☐ No ☐ 4. Fever Yes ☐ No ☐

5. B.P: …… 6. Pulse: ……/min

7. Organomegaly: Liver ☐ Spleen ☐ Kidney ☐

8. Oedema: Yes ☐ No ☐

## ***E- Result:***

### ***E1: Blood Film:***

Thick B.F……Thin B.F…….

***E2:Optimal test for malaria:*** ……

### ***E3: Urine analysis:***

1. Color…….. 2. Reaction ……3. Albumin…….

4. Sugar……..5. Acetone…….. 6. Bile pigment……..

7. Pus cell……HPF 8. RBCs……HPF

9. Epithelial cell……..HPF 10.Crystals……

11. Casts……. 12.Ova…….

12. Others ……..

### ***E4: Blood Chemistry:***

#### ***(i) RFT:***

1. B. urea…… mg/dl. 2. S. Creatinine……mg/dl

3. S. Sodium……mmol/l. 4. S. Potassium…….mmol/l

5. S. chloride ……. mmol/l

#### ***(ii) LFT:***

1. T. protein…… g/dl 2. S. albumin…… g/dl

3. S. globulin…… g/dl 4. S. Total Bilirubin……..mg/dl

5. S.Direct Bilirubin….mg/dl. 6. S. Indirect Bilirubin …..mg/dl

7. S. SGOT:…….. IU/ml 8. S. SGPT: …….. IU/ml

9. S. ALP: …….IU/ml 10. S. G.G.T……IU/ml

### ***E5: haematological prameters:***

Platlate count …… MPV……

PDW……

## استمارات الموافقة الأخلاقية

### استمارة موافقة المريض على المشاركة بالبحث

**يجب أن تكتب الاستمارة بلغة عربية واضحة ومفهومة وتحتوي على الفقرات الآتية**

**أسم الباحث.....**

**عنوان الباحث.....**

**مكان إجراء البحث.....**

**أنت مدعو للمشاركة ببحث علمي سريري في ( .....).**

**يرجى أن تأخذ الوقت المناسب لقراءة المعلومات الآتية بتأنٍ قبل أن تقرر إذا ما كنت راغباً بالمشاركة أم لا. وبإمكانك طلب مزيداً من**

**الإيضاحات أو المعلومات الإضافية عن أي أمر مذكور بالاستمارة أو عن الدراسة من طبيبك.**

**1- وصف مشروع البحث وأهدافه ومساره:**

**2- الفوائد الايجابية المحتملة للمشارك التي قد تنتج من هذا البحث.**

**3- التأثيرات السلبية أو الأعراض الجانبية المحتملة التي يتعرض لها المشارك.**

**وفي حال موافقتك على المشاركة في هذه الدراسة سيبقى أسمك قيد الكتمان. ولا يسمح لأي شخص حق الاطلاع على الملف الطبي**

**الخاص بك .**

### وثيقة الموافقة التحريرية

**لقد أوضحت للمشارك بالتفصيل البحث وطبيعته ومجرياته وفوائده المحتملة وسلبياته المحتملة أيضاً. وأجبت عن كل استفساراته وأسئلته**

**بوضوح . وسأعلم المشارك بأي تغييرات في مجريات البحث أو فوائده أو سلبياته حال حصولها في أثناء البحث.**

**أسم الباحث ..... التوقيع التاريخ / /**

### موافقة المشارك

**لقد اطلعت على استمارة الموافقة وأدركت مضمونها وتمت الإجابة عن جميع الأسئلة التي تجول في ذهني. وبناءً عليه فأني حراً ومختاراً**

**أوافق على المشاركة بالبحث. وفهمت أن الباحث / الدكتور ( ) وزملاؤه ومساعديه**

**سيكونون مستعدين للإجابة عن أسئلتي المستقبلية. وباستطاعتي الاتصال بهم على رقم الهاتف ( ). كما أعلم تماماً**

**بأنني حر في الانسحاب من هذا البحث متى شئت ولو بعد الموافقة التحريرية ومصادقتها من دون أن يؤثر ذلك على العناية الطبية المقدمة**

**لي. وسيزودني الباحث بنسخة من هذه الموافقة الخطية**

**أسم المشارك..... التوقيع التاريخ /**

## 6.2.2: Study area (River Nile state)