ASSESSMENT OF SOME ENZYMES AND HAEMATOLOGICAL PARAMETERS IN MALARIA PATIENTS, RESIDENT IN MINNA AND ITS ENVIRONS

BY

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DECLARATION

I, Abubakar, Asmau Niwoye (M.Tech/SSSE/2007/1644) hereby declare that this project work titled *"Assessment of Some Enzymes and Haematological Parameters in Malaria Patients, Resident in Minna and it Environs"* was carried out by me under the supervision of Dr. A.A. Jigam of the Department of Biochemistry, Federal University of Technology, Minna, and has never been presented elsewhere for the award of any degree. All the materials and other sources of information used in this work have been duly cited and referenced.

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CERTIFICATION

This thesis entitled: *"Assessment of Some Enzymes and Haematological Parameters in Malaria Patients, Resident in Minna and Its Environs"* by Abubakar, Asmau Niwoye (M.Tech School of Science and Science Education, Reg. No.: M.Tech/SSSE/2007/1644) meets the regulation governing the award of the degree of Master of Technology of the Federal University of Technology Minna and is approved for its contribution to scientific knowledge and literary presentation.

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DEDICATION

This project is dedicated to Almighty Allah for granting me good health and sustaining me throughout the course of study. I say Alhamdullilah. And my caring, loving and ever supporting husband Alhaji Mohammed Nma Abubakar.

ABSTRACT

The activities of some serum enzymes which included serum glutamate pyruvate transaminase (SGPT), serum glutamate oxalate transaminase (SGOT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP) and glucose-6-phosphate dehydrogenase (G-6-PD) with haematological parameter packed cell volume (PCV) and Haemoglobin (HB) were assayed in one hundred and one malaria patients attending the outpatient unit of the General Hospital Minna, Nigeria. The results obtained indicated that the activities of serum glutamate pyruvate transaminase (36.9±2.1iu/L), serum glutamate oxalate transaminase (35.0±2.2 iu/L), alkaline phosphatase (45.7±1.7 iu/L) and glucose-6-phosphate dehydrogenase (0.012±0.008 iu/L) in malaria patients were significantly difference ($P<0.05$) from the values in controls SGPT (27.50±2.6 iu/L), SGOT (21.9±1.3 iu/L), alkaline phosphatase (35.5±2.5 iu/L) and G-6-PD (0.015±0.009). lactate dehydrogenase activities were comparable between the patients and healthy subjects. The results from males alone, exhibited a similar trend as that for the general population above i.e significant ($P<0.05$) variations in SGPT, SGOT, ALP and G-6-PD. lactate dehydrogenase actiivites were however comparable between male patients and male controls. Comparison of enzymes values between females alone demonstrated significant difference ($P<0.05$) in the activities of SGPT, SGOT, LDH and G-6-PD but not ALP. Values for females and males showed that only SGOT and SGPT were not significant ($P>0.05$). The haematological parameters PCV and HB were generally higher in the controls as compared with malaria patients and this was irrespective of the gender. Therefore it is concluded that enzymes activities are mostly elevated in malaria patients and red blood cell are also destroyed depending on the level of parasitamea.

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CHAPTER ONE

1.0 INTRODUCTION

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

Malaria is essentially a tropical disease occurring in regions between latitude 62°N and 40°s with altitude of 1,500m. This region is formed mainly within the tropics and subtropics and this makes malaria endemic in the zone (Walter and Davies, 1976). There are two epidemiological extremes of malaria known as stable and unstable. Epidemiological factors that make malaria endemic in the tropics include climatic factors (Relative humidity, rainfall level, mean temperature between 18 – 29°C, altitude) and socio-economic factors. All these have effects on the availability of vectors which maintains the transmission of malaria (Bulter, 1996).

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

Virtually, no reliable statistic exists in Africa. However, an estimated 90% of the total malaria incidence and deaths is said to occur in Africa, particularly amongst pregnant women and children (Luxem Burger, 1997; Carrington, 2001). Malaria is the most widespread parasitic disease and one of the vectors borne disease placed on the world health organization's Disability Adjusted Life Years (DALYS). List, with

falciparum malaria responsible for more death than any other type of the disease. (Carrington, 2001).

Malaria can be transmitted by vector transmission, (Anderson, *et al.,* 1981), blood transfusion (Strickland, 1991) and Congenital transmission (Ezechukwu, *et al.,* 2004). The vector for malaria parasite is the female anopheles mosquito (Cheesebrough, 1998). Malaria is a protozoan infection caused by the parasite plasmodium. There are four species of the parasite that infect man, namely, *P. falciparum, P. malaria, P. ovale* and *P. vivax* with *P. vivax* and *P. falcipium* being the most common (Mc gee, *et al.,* 1992).

Malaria interferes with three organs in the body namely; the brain, liver and kidney (Edington, 1967). The inversion of the liver cells by the parasite can cause organ congestion, sinusoidal blockage and cellular inflammation (Jarike, *et al.,* 2002) when this happens, the parenchyma (transaminases) and membranous alkaline phosphatase and gamma glutamyl transpeptidase enzymes leak out to find their way into the circulation leading to increased enzyme activity (Burtis *et al.,* 2001). Patients with normal G-6-PD activity may be more susceptible to malaria because of the less oxidative stress which enhance parasite growth (CESSC, 1974) and the LDH activity also correlates with the parasitemia level (Uzoegwu *et al.*, 2003).

It has also been shown that some other pathological condition like hepatitis, athrosis, myocardial infarction and muscular dystrophy also lead to increased activity of liver enzymes (Elles *et al.,* 1978). However, the levels of increase in the enzyme activity of the liver vary with the causative factors (Kim, *et al.,* 1991).

Malaria causes such catastrophes as maternal and infant death and abortion. Susceptible groups are children and adults who have lost or never acquired immunity. It precipitates such terribly mutilating affiliation (in children) as cancrum oris and

numerous complication such as anaemia, pulmonary oedema, renal failure and coma which may be fatal (Eze and Mazeli, 2001).

1.1 ENZYMES

Enzymes are biological catalysts that speed up the rate of chemical reactions without being altered. They act as specific regulators of metabolism. Enzymes, are important markers which helps us to denote disease conditions. For example, intracellular enzymes of which in health only small amounts are present in blood plasma but when an organ is diseased, a greater amount of it's enzyme may escape so that there is increase, often marked by their activity in the plasma. Some distinct features of enzymes include; lower activation energy, mild temperature condition ($37^{o}C$), they play regulatory role, they exhibit high specificity, the rate of enzymatic action is proportional to the amount of enzyme (CESSC 1974). Below are the enzymes to be accessed.

1.1.1 Alkaline Phosphatase

Alkaline phosphatases are a group of enzymes found primarily in the liver (Isoenzyme ALP-1) and bone (Isoenzyme ALP-2). There are also small amounts produced by cells lining the intestines (Isoenzyme ALP-3), the placenta, and the kidney (in the proximal convoluted tubules). What is measured in the blood is the total amount of alkaline phosphatases released from these tissues into the blood. As the name implied, this enzyme works best at an alkaline pH (a pH of 10), and thus the enzyme itself is inactive in the blood. Alkaline phosphatases act by splitting off phosphorus (an acidic mineral) creating an alkaline pH (KIM et al; 1991).

$$\text{Phenolphosphate} \xrightarrow[\text{pH 10}]{\text{ALP}} \text{phenol} + \text{phosphate}$$

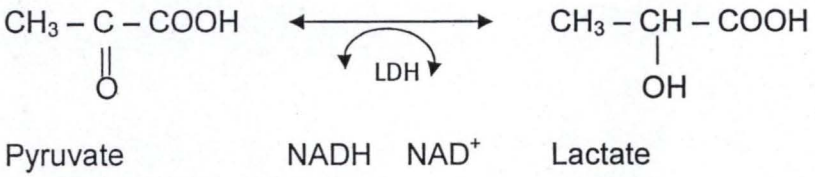
1.1.2 Glucose 6-Phosphate Dehydrogenase

(ii) Glucose 6-phosphate dehydrogenase (G6PD or G6PDH) is a cytosolic enzyme in the pentose phosphate pathway, a metabolic pathway that supplies reducing energy to

cells (such as erythrocytes) by maintaining the level of the co-enzyme nicotinamide adenine dinucleotide phosphate (NADPH). The NADPH in turn maintains the level of gluthathion in these cells that helps protect the red blood cells against oxidative damage of greater quantitative importance is the production of NADPH for tissues actively engaged in biosynthesis of fatty acids / or isoprenills such as the liver, mammary glands, adipose tissue and the adrenal glands (Cappellini, *et al,* 2008).

1.1.3 Lactate Dehydrogenase (LDH)

Lactate dehydrogenase catalyses the interconversion of pyruvate and lactate with concomitant interconversion of NADH and NAD^+. It converts pyruvate the final product of glycolysis to lactate when oxygen is absent, or in short supply and it's perform the reverse reaction during the cori cycle in the liver. At high concentrations of lactate, the enzyme exhibit feedback inhibition and the rate of conversion of pyruvate to lactate is decrease. It also catalyses the dehydrogenation of 2-hydroxybutyrate, but it is a much poorer substrate than lactate. There is little to no activity with beta-hydroxybutyrate.



The lactate dehydrogenase enzyme is widely distributed in the heart, liver, muscle and kidney. (Heffner, *et al;* 1997).

1.1.4 SGPT and SGOT Enzymes (Transaminases)

Alanine transaminase (ALT) also called serum Glutamic pyruvic Transaminase or Alanine Aminotransaminase (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 (ULN).

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 deviated in acute liver damage. It is also present in red cells, cardiac and skeletal muscle. The ratio of AST – ALT is useful in differentiating between causes of acute hepatitis. SGPT (Ellis, *et al;* 1978).

1.2 Aims and Objectives

The aims of this project include, Screening of blood for malaria parasite, Determination of ALP, SGOT, SGPT, Glu-6-PD and LDH levels in malaria and Screening of haematological parameters, PCV and haemoglobin.

The objectives of this work are;

i. To ascertain whether there is any correlation between enzyme levels and plasmodial infection;
ii. To obtain baseline values for each enzymes in malaria;
iii. To assess the use of enzyme assay in malaria diagnoses and treatment.

1.3 Justification

i. The endemic nature and resistance problem of malaria deem it fit for a continual research.
ii. The significant role of enzymes can help in new and further drug development for malaria.

CHAPTER TWO

LITERATURE REVIEW

2.1 HISTORICAL BACKGROUND

The history of malaria is as old as man's history. It is the most ancient infection known and clearly defined, due to its peculiar clinical nature (Polozok, *et al.*, 1989). The earlier record of malaria was 5th century BC (460 – 377BC) as found in the record of the Hippocrates works. In the 17th century, the Italians named the disease malaria (bad air) because of its association with the ill-smelling vapours from the swamps in the vicinity of Rome. Malariology began in 1640 when Huandel vego employed tincture of Cinchona tree back for malaria treatment (Harrison, 1978). The German scientist, Meckel, (1947) detected leukocytes – Macrophages and brownish pigments associated with malaria. Alphose Laverai (1880), an army surgeon in Algeria described malaria parasite in human blood cell (RBC) while Golgi (1885) the presence of *plasmodium vivax* and *plasmodium malaria* (Ronald Ross 1897) established that mosquitoes serves as vectors of human and avian malaria. Grassi Bignami Bastianelli confirmed this in 1898. Manson (1990) confirmed the mosquitoes genus as anopheles. Sakharou, (1819); Marchafava and cell (1890) described *P. falciparum (*All as cited in Polozok *et al.*, 1989 *).*

In Africa, Stephens (1922) discovered fourth type of the causative agent of malaria (WHO, 1983).

The major history of malaria in the 20th century has included the efforts directed towards understanding of plasmodium physiology and biochemistry, controlling it's mosquito vector and developing chemotherapeutic agents (Smyth, 1996). It was for example, demonstrated in the 1960s that resistance of plasmodium *falciparum* among West African negroes is associated with the presence of

haemoglobin S. (Hbs) in their erythrocytes. Hbs differs from normal haemoglobin – A (HbA) by a single insoluble amino acid, valine which has replaced the soluble glutamate at position 6 of the β haemglobin chain. Consequently, these erythrocytes responsible for sickle cell disease have a low binding capacity for oxygen. Because plasmodium has a very active aerobic metabolism, it cannot thrive within these erythrocytes. Neel (1949) proposed that individuals heterozygous to the sickling gene enjoy advantages not shared by either normal heterozygous or sickle cell homozygote. Infact, Allison (1956) found that people with sickle traits are protected against the most lethal form of malaria – a case of balanced polymorphism. The incidence of malaria and the frequency of the sickle gene in Africans are correlated (Stryer, 1980).

Similarly, the significance of such historical findings is that of transformation (Mutation) in human house keeping gene called Glucose – 6 – Phosphate dehydrogenase (G6PD) (Connor, 2001) that brought about protection against infection specifically *P. falciparum* with individuals with the deficiency. (Robinson, 2000), haemoglobin F in the foetus are also associated with limited protection in human against malaria and are manifested in human as a result of genetic transformation (Cheesebrough, 1991) such an advent may possibly have historical relationship with malaria outbreak.

Malaria is an infectious disease caused by a parasite (*plasmodium*) transmitted from human to human by the bite of infected female anopheles mosquito (Aikawa, *et al.*, 1980).

Table 1.0: Classification Of Malaria Parasite (Leach and Jeffery, 1975)

Phylum — **PROTOZOA**

Unicellular organisms

SPOROZOA | SARCODINA | CILIOPHORA | MASTIGOPHORA | UNCERTAIN STATUS

Sub phylum — SPOROZOA

No special locomotor apparatus alternate asexual cycle

Class

TELOSPORIDEA

Sexual spore (Oocyst) simple

SARCOSPORIDEA

Sexual spore (Oocyst) simple

Sub Class

COCCIDIA | HAEMOSPORIDA

Sexual & asexual generations in different hosts parasitic in fixed tissue cells & red blood cells of vertebrate host

Family

PLASMODIIDAE

Include human parasite produce pigment in asexual cycle in red blood cells of vertebrates produce gametocytes in red blood cells of vertebrates sporogony (sexual cycle) in invertebrate

Genus

PLASMODIUM

Schizogony (asexual cycle) in red blood cells & other tissue cells of vertebrate host pigment derived from haemoglobin of infected cells. Gametocytes develop in some red cells. These undergo sporogony (sexual cycle) in various species of mosquito sporozoites produced in mosquito, infective to vertebrate host. All malarial parasites included in this genus

Species

P.vivax	*P.malaria*	*P.falciparum*	*P.ovale*	*Others*
Causes benign Tertian Malaria	Causes Quartan Malaria	Causes malignant Malaria	Causes ovale Malaria	Parasite numerous species of animals & birds

Table 2.0: Some Species of Plasmodium

Animals	Plasmodium spp
Human	*P. falciparum; P. vivax; P. ovale; P. malariae*
Simian (Monkeys, Baboons)	*A. Old World: P. cynomolgi; P. gonderi; P. inui; P. coatneyi; P. fragile; P. fieldi; P. simiovale; P. knowlesi; P. shortii; P. eyelesi* *B. New World: P. simium; P. brasilianum*
Rodent	*P. berghei; P. yoelli; P. anamaluri; P. vinckei; P. argyptensis; P. artheruri; P. landauae; P. pulmophilum; P. bootiati; P. watteni; P. chabaudi*
Avian	*P. gallinaceum; P. relictum; P. cathemerium; P. lophurae; P. elongatum; P. circumflexum; P. juxtanucleare*
Unknown status	*P. pitcheci; P. reichenovi; P. rhodaini; P. schwetzi*

Source: Bruce-Chwatt, 1986

Most *Plasmodium* species are parasites of birds, rodents, primates, reptiles and other mammals such as squirrels and bats (Table 2.0). They are also found in amphibians but have not been much studied in most of the aforementioned animals (Smyth, 1996). Some species are useful in laboratory studies of immunity, physiology, etc, but their importance to human medicine is for the most part unassessed. Usually they are potential disease agents, at least to the individual who may be exposed under unusual circumstances (Bruce – Chwatt, 1986).

2.2 AETIOLOGY OF MALARIA

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

Man is said to be with several types of malaria parasites infectious in monkey such as *plasmodium knowlesis P. cynomolgi bastianeli, p. nui, p. simium* and p.*shorti.* They can be contracted both experimentally and under natural condition, with possible subsequent transmission to another man via mosquitoes (Polozolk, 1989).

2.3 HUMAN PLASMODIA

Excluding simian malaria, there are four species of plasmodium that infect man and result in four kinds of malaria fever:

i. *Plamodium* vivax benign, simple or tertian malaria.

ii. *Plamodium falciparum*, aestivo – autumnal, malignant tertian pernicious quotidian, subtertian or tropical malaria.

iii. *Plamodium malariae*; quartan ague, or quartan malaria.

iv. *Plamodium ovale*; ovale tertian malaria

The species differ morphologically, immunologically, in geographical distribution, relapse pattern and in drug response. Among those species mentioned above, *Plamodium vivax* shows the widest distribution, being prevalent throughout the tropics and many temperate regions. *Plamodium Vivax* malaria is characterized by relapses: reappearances of symptoms after a latent period of up to 5 years, as is infection with *Plamodium ovale*, which occurs chiefly in tropical Africa (Bruce-Chwatt, 1980). The relapses are due to the sudden activation of hypnozoites (sleeping merozoites) in liver cells. *Plamodium falciparum* is most common in tropical and sub-tropical areas and causes the most dangerous malignant from of malaria, which fortunately does not have relapses. *Plamodium malariae* is widely distributed but much less common than *Plamodium vivax* or *Plamodium falciparum*. Although, *falciparum* malaria or *malariae* malaria do not show relapses, they are subject to "recrudes-cences": repeated manifestations of infection after a short talent period between 3 months and 1 year (Wernsdofer and Werndorfer, 1988).

2.3.1. *Plamodium falciparum*

It is the most important malaria parasite; the disease it produces is acute and often terminating fatally. It is a significant cause of abortion or still birth and even death of non-immune pregnant women. It causes 50 percent of all the malaria cases throughout the world. It distribution is restricted to warm and tropical countries. The distinct features of it's lifecycle may be summarized as follows:

(i) It can attack erythrocytes of all ages indiscriminately so that a high density of the parasite can be rapidly reached. In severe cases up to 48 percent of the red cells may be parasitized.

(ii) Multiple infections i.e. polyparasitism resulting in several ring forms in a corpuscle are not uncommon.

(iii) The later stages in the asexual cycle, that is the schizonts, do not occur in the peripheral blood as in other forms of malaria, except in severe cases, thus only ring and crescent are found in blood films.

Having prolonged for 24 hours, the rings forms and older trophozoites show a tendency to clump together like resetting and adhere to the visceral capillary walls (sequestration) and become caught up in the vessels of the heart, intestine, brain (convulsion develop due to anoxia) or bone narrow in which the later asexual cases are completed. This phenomenon together with the fact that the subtertian malaria is more toxic, are the principal reasons why this type is so dangerous.

(iv) Exo-erythrocytic forms do not persist in the tissues and hence relapses do not occur (Warrell, *et al.*, 1990).

2.3.2 *Plamodium vivax*

This plasmodium causes the benign tertian form of malaria, which causes about 43° percent of the total cases in the world and has the widest geographical distribution. Although mostly not life threatening but can cause severe, acute illness, the following are observed in its lifecycle:

(i) The rate / degree of its infection is low, only the young immature corpuscles i.e. reticulocytes are being attacked; almost 2 percent of the erythrocyte are parasitized;

(ii) The periodicity of the asexual cycle is synchronized;

(iii) Hypnozoites develop in the liver, so that relapses may occur.

Plamodium vivax morphological features is very similar to that of a sub specie – p – cynomolgi, the resemblance is so similar such that the differentiation is difficult (Coatney, 1968).

2.3.3. *Plamodium ovale*

This species is rarely encountered, it is confined essentially to the tropics and sub-tropics although reported from many continents: the fever it produces is called Ovale tertian which is usually milder than the benign tertian of p. vivax.

It special features include:

(i) In most stages, it resembles *Plamodium malaria* morphologically;

(ii) The changes that occur in the erythrocytes in general are similar to those produced by p. vivax but schnuffner's dot appear. Considerably earlier (in the ring stage) and are coarser and more numerous.

(iii) In the Oocyst the pigment granules are usually characteristically arranged in two rows crossing each other at right angles.

(iv) Hypnozoites develop in the liver such that relapse can occur.

2.3.4. Plamodium Malaria

It is a relatively rare parasite that produces quartan malaria which is responsible for about 7 percent of the malaria in the world. It special features are:

(i) The infected erythrocytes are not larger than the uninfected ones infant sometimes even smaller.

(ii) Mature erythrocytes are attacked and rarely reticulocytes so that the density of parasites is very low; about 0.2 percent of erythrocytes are parasitized.

(iii) It is usually difficult to distinguish between a large trophozoite and an immature gametocyte.

2.4 LIFE CYCLE OF PLAMODIUM

So far, all plasmodia of animals, including man, spend a part of their life in vertebrate and part in a mosquito host it even suggested that some species of plasmodia of reptiles and bats may have arthropod hosts other than mosquitoes thought not yet confirmed (Smyth, 1996).

In the life cycle of most species of plasmodia, there are three phases of development; all phases are not known for every species. The first phase is the exo – erythrocytic (EE) stage in the tissues, usually in the liver.

The second is the erythrocytic schizogony in the erythrocytes. The third stage is the sexual process which begins with the development of gametocytes in the vertebrate host and continuing with the differentiation of micro and macro gametes, fertilization and sporogony in the mosquito.

Generally, the pattern of the life of human plasmodia is the same but there are some important physiological differences, which is usually reflected in the nature of the disease, produced. Many of this characteristic features are sufficiently definite to be used as criteria for identification in blood films, though species differentiation may not be possible in the earliest stages (Smyth, 1996).

Available knowledge to date indicates the plasmodia of man develop only in female mosquitoes of the genus, anopheles and not in any other arthropod. Almost any species can be infected with *plasmodia* in the laboratory, but many species are poor vectors and not natural ones. There are more than 2,500 species of mosquito with only a minority feeding on human blood of the approximately 400 species of anopheles throughout the world; only about 60 are vectors of malaria under natural conditions, some 30 of which are major importance (Jones, 2003). The female *anopheles* mosquito drinks blood apparently to fuel the production of eggs.

In each geographical area, there are usually not more than three anopheles species that can be regarded as important vectors. (Bouree, *et al.,* 1993). For mosquito to be an efficient vector, it must possess certain characteristics:

(a) Susceptibility to infection and physico-chemical and nutritional characteristics suitable for the development of plasmodia;

(b) It must bite man in preference to animals e.g. a darlingi in south Africa.

(c) It must not be shy of human habitation.

(d) Its life span must be sufficiently long to permit sexual development of the plasmodium.

2.4.1 Asexual Development of Plamodium parasites in the Vertebrate Host

When an infected mosquito bites man, numerous sporozoites (several hundreds) may be injected. Since a mosquito usually feels about with its proboscis until it strikes a small capillary, the sporozoites are probably injected directly into the blood stream.

Here, they remain about 30 minutes and then disappearance from the blood stream, some of the sporozoites are destroyed by phagocytes but some enter the hepatocytes in the liver through the Kupffer cells, in the liver, they multiply rapidly by schizogony, a process referred to as pre-erythrocytic schizogony. When schizogony is completed the merozoites (about 30,000 per single sporozoite) are released from the schizont invade the erythrocytes and the classical erythrocytic cycle begins.

In the human malaria species, it was originally thought that there were two successive pre-erythrocytic cycles, but it is presently known that P.*vivax* and P.*ovale*, some injected sporozoites may differentiate into stages termed hypnozoites (Greek hypnos means sleep, zoites means animal) which may remain dormant in the

liver cells for some time only to undergo schizogony causing relapse of disease when the red cells are invaded (Smyth, 1996).

It was formerly thought that the merozoites released from a schizont directly 'penetrated' the membrane of a red blood cell and then developed. Largely owing to the work of Aikawa *et al.,* (Aikawa, 1980; Aikawa and Seed, 1980). It has been shown that the merozoites enter erythrocytes by endocytosis. The inhibition of this key event can be regarded as a crucial goal in the development of a malarial vaccine (Parkins, 1989). When entry into the host cell is completed, the merozoites is surrounded by a parasitophorus vacuole that originated from the erythrocyte membrane. This grows with the developing parasite and is retained until the formation of the next generation of plasmodium merozoites (Aikawa, 1980).

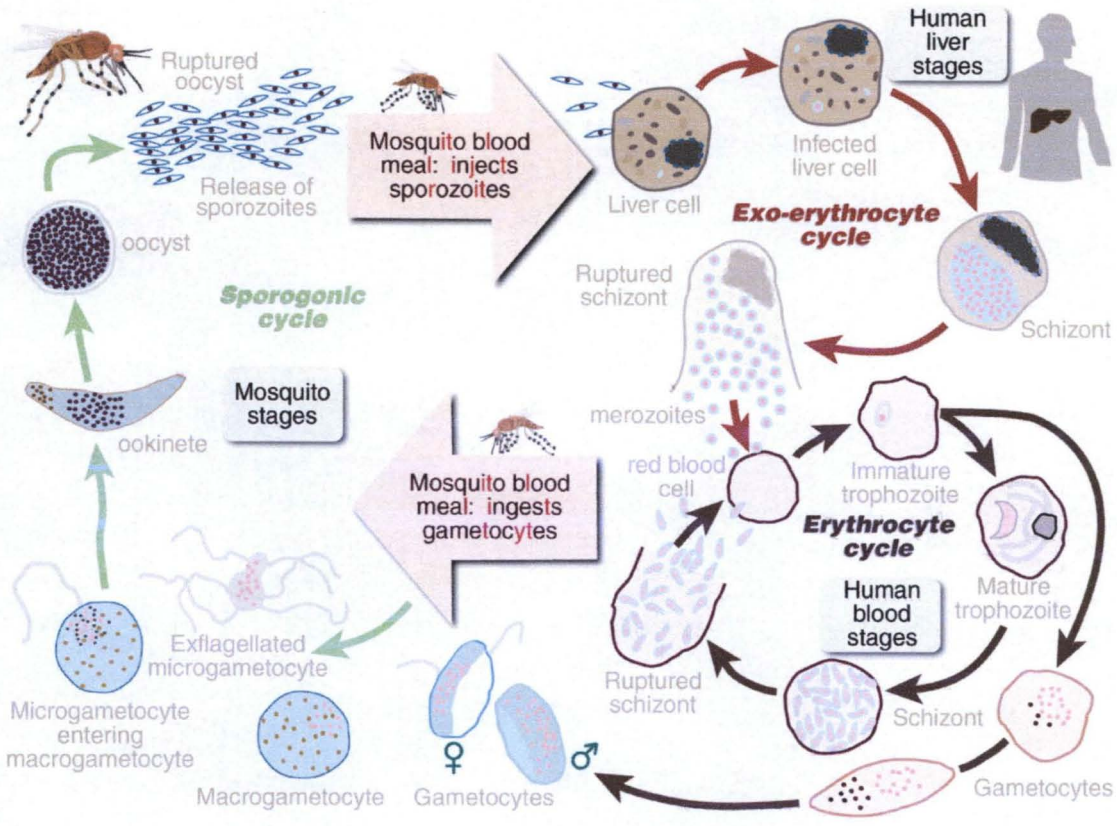


Figure 2.1: Life Cycle Of Mosquito. Source: CDC, (2004).

2.4.2 Erythrocytic Schizogony in malaria

Within an erythrocyte, the parasite is first seen microscopically as a minute strand of chromatin surrounded by scanty protoplasm. The uninucleate plasmodium gradually becomes ring - shaped and is known as a ring or trophozoite, It grows at the expense of the erythrocyte and assumes a form differing widely with the species but usually exhibiting active pseudopodia. Pigment granules of haemozoin appear early in the growth phase. As the chromatin begins to divide, the parasite is known as a Schizont. The dividing chromatin tends to take up peripheral positions. and a small portion of cytoplasm gathers around each. The mature schizont is often known as a Segmenter. The infected erythrocyte ruptures and releases a number of merozoites, which attack new corpuscles, thus repeating the cycle of erythrocytic schizogony. The infection about this time enters the phase of patent parasitaemia with parasites detectable in blood smears.

In some species, merozoites show a distinct prediction for erythrocytes of a certain age. Many avian species attack almost exclusively the young erythrocytes. In the human plasmodia, the merozoites of *P. vivax* attack young immature corpuscles (reticulocytes), those of P. malarial attack the older ones and those of *P. falciparum* indiscriminately enter any available. Schizogony may continue for many months or even years; some cases of *P. malariae* have been recorded in which the infection seems to have persisted for 30 - 40 years (Garnham. 1988).

All schizonts release their contained merozoites within a period of several hours together with the haemozoin pigment and other waste products (toxins) and there is a sudden paroxysm of fever in the host characterized by a marked rise in temperature. This periodicity in reproduction is remarkable human and avian species

being particularly regular. The length of the cycle is usually some multiple of 24 hours. In *P. vivax, P. ovale* and *P. falciparum* it is 48 hours and in *P. malarie*, it is 72 hours, but in some monkey species (e.g *P. knowlesi*) and some avian species (e.g. *P. cathemerium*) it is 24 hours.

2.4.3 Formation of Gametocytes (Gametocytogenesis)

Some merozoites on entering red cells become sexual gametocytes, instead of asexual schizont. Male gametocytes are termed microgametocytes, and female, macrogametocytes. The stimulus that induces a merozoite to undergo gametocytogenesis, instead of schizogony, is largely unknown. It is generally held to be related to stress due to rising asexual parasitaemia, the effects of drug suppression, nutrient immunity to the asexual stages; reliable supporting evidence is however lacking (Sinden, 1983b). The process of invasion of an erythrocyte destined to become a gametocyte appears to be the same as that of a merozoite destined for asexual development. Thus the gametocyte also becomes surrounded by two continuous membranes, the host cell membrane and the parasitophorous vacuole membrane.

Although both male and female gametocytes possess mitochondria, the microgamete itself lacks one (Sinden, 1983a), when gametocytes are ingested by a mosquito, the cells rapidly undergo gamete production, generally referred to as 'exflagellation', although strictly speaking, use of this term should be restricted to one phase of microgametogenesis (Carter, *et al.*, 1988). The process is readily carried out in vitro and a number of factors stimulate this process e.g. presence of serum or bicarbonate ions. This does not necessarily indicate that the same factors affect this process invivo.

The formation of female gametes - macrogametogenesis - involves little more than their escape from the host cell with relatively few structural changes. The formation of male microgametes - microgametogenesis - is however, a somewhat explosive phenomenon and the ultra structural changes are rapid and stunning. Within 15 seconds the cytoplasmic microtubule organizing centre becomes transformed into two orthogonal planar tetrads of kinetosomes upon which axonemes immediately condense (Sinden, 1983a). A complex cytoplasmic reorganization follows, involving rapid nuclear divisions which result in the production of eight motile haploid gametes.

The free male gamete normally has a single kinetosome. Sinden and Croll (1975) recognized three phases in microgametogenesis: i. Maturation
ii. Exflagellation and iii. escape

In *P.y. nigeriensis*, maturation takes about 7 minutes and exflagellation less than 1 minute, but the escape of microgametes may continue for 30 - 40 minutes. Gamecytogenesis has also been described at the ultra-structural level in P. *falciparum* (Sinden, 1982).

After a microgamete fertilizes a macrogamete, the resulting zygote develops into a mobile elongated ookinate. This penetrates the gut wall of the mosquito between the cells and develops as an oocyst between the epithelium and the basement membrane. The oocyst matures in 10 - 20 days depending on temperature, species and perhaps individual mosquitoes. Growing to a body 50 - 60μm in size. The chromatin divides repeatedly until there are hundreds of tiny nuclear masses, and the cytoplasm follows suit resulting in the production of enormous numbers of threadlike Sporozoites up to one thousand. When mature, an

oocyst bursts and the released sporozoites migrate to the salivary glands where they become intracellular or intracellular organisms, or remain free in the ducts.

There is evidence that the morphological cycle of maturation of gametocytes corresponds to the period of infectivity to mosquitoes (Hawkings *et al;* 1968). This period of high infectivity of the gametocytes corresponds closely to the limited period during which vector mosquitoes normally bite, It is clear that such a synchronization of gametocyte and mosquito cycles would have a marked selective advantage (Smyth, 1996).

Although avian, rodent and simian species of malaria differ somewhat in certain details of morphology and lifecycle, these differences are not very great and the general picture outlined above can be accepted as the basic pattern of the life cycle (Garnham, 1966).

2.5 GENETIC RESISTANCE AND IMMUNITY TO MALARIA

2.5.1 Sickle Cell Diseases and immunity to malaria

The most studied influence of malaria parasite upon the human genome is a hereditary blood disease, sickle cell trait on both genes causes disease, but those only partially affected (carrying only one gene with the trait) by sickle – cell have substantial protection against malaria.

In sickle cell disease, there is a mutation, in this HHB gene, which encodes the beta-globin subunit of haemoglobin. The normal allele encodes a glutamate at position six of the beta-globin protein, whereas the sickle cell allele encodes a valine. This change from a hydrophilic to a hydrophobic amino acid encourages binding between haemoglobin molecules, with polymerization of haemoglobin deforming red

blood cells into a sickle shape. Such deformed cells are cleared rapidly from the blood, mainly in the spleen, for destruction and recycling.

In the merozoites stage of its lifecycle, the malaria parasite live inside red blood cells, and its metabolism changes the internal chemistry of the red blood cell infected cells normally survive until the parasite reproduces, but if red cells contains a mixture of normal and sickle haemoglobin, it is likely to become deformed and destroyed before the daughter cell emerge. Thus, individual heterozygous for the mutated allele, known as sickle cell trait may have a low and usually unimportant level of anaemia, but also have to a great extent reduced chance of having malaria. Thus, a classic example of heterozygote advantage, Individuals homozygous and in traditional societies rarely lives beyond adolescence. However, in populations, where malaria is endemic the frequency of sickle cell genes is around 10%. The existence of four haplotypes of sickle type haemoglobin suggest that this mutation has emerged independently at least four times in malaria endemic areas, further demonstrating its evolutionary advantage on such affected regions. There are other mutation of the HBH such as HBE and HBC, these two mutation are also capable of conferring similar resistance to malaria infection. The mutations are common in southeast and western Africa respectively. (Robinson, 2000).

2.5.2 Thalassaemia and immunity to malaria

Another well documented set of mutations found in the human genome associated with malaria are those involved in causing blood disorders known as thalassaemia. Studies in sardine and pupa new guinea have found, that the gene frequency and malaria endemicity in the α+ from α-thalassemia. Presumably, these gene have also been selected in the course of human evolution.

2.5.3 Duffy Antigens and immunity to malaria

The Duffy antigens are antigens expressed on the erythrocytes and other cells in the body acting as a chemokine receptor. The expression of Duffy antigens on the blood cells is encoded by fy genes (fya, fyb, fyc, etc) *plasmodium vivax* malaria uses the Duffy antigen to enter blood cells. However, it is possible to express no Duffy blood cells. However, it is possible to express no Duffy on the erythrocytes (fy-/fy-) this genome confers resistance to *P.vivax* infection. The genotype is very rare in European, Asian and American populations, but is found in almost all the indigenous population of west and central Africa (Carter, *et al.,* 2002). This is thought to be due to very high exposure to *P.vivax* in Africa in the last few thousand years.

2.5.4 Glucose-6-Phosphate Dehydrogenase and immunity to malaria.

Glucose-6-phosphate dehydrogenase (G6PD) is an enzyme that normally protects from the effects of oxidative stress in red blood cells. However a genetic deficiency in this enzyme results in increased protection against severe malaria. (Capellini *et al;* 2008)

2.5.5 Human Leukocyte Antigen and interleukin-4 and immunity to malaria.

HLA-B53 is associated with low risk of severe malaria. This MHC- class 1molecule presents liver stage and sporozoite antigens to T-cells. Interleukin-4 encoded by IL4, is produced by activated T cells and promotes proliferation and differentiation of antibody producing B-cells. A study of the Fulani of Burkina Faso who have both fewer malaria attacks and high levels of anti-malaria antibodies than do neighboring ethnic groups, found that the IL4-524 T allele was associated with

elevated antibody levels against malaria antigens, which raises the possibility that thus might be a factor in increased resistance to malaria (Verraf *et al.*, 2004).

2.5.6 Regional Resistance to malaria in South Africa

The lowest Himalaya foothills and inner Terai or Boon valleys of Nepals and India are highly malarial due to a warm climate and marshes sustained during the dry season by groundwater percolating down from the higher hills. Malaria forests were intentionally maintained by the rulers of Nepal as a defensive measure. Humans attempting to live in this zone suffered much higher mortality than at higher elevations or below on the drier Gangetic plain.

However, the tharu people had lived in this zone long enough to evolve resistance through multiple genes. Medical studies among the tharu and non-tharu population of the Teria yielded the evidence that the prevalence of cases of residual malaria is nearly seven times lower among tharu. The basis for their resistance to malaria is most likely a genetic factor. Endogamy along cost and ethnic lines appear to have confined these to the tharu community (Terrenato *et al* 1988). Otherwise, these genes probably would have become nearly universal in South Asia and beyond because of their considerable survival value and the apparent lack of negative effects comparable to sickle cell anaemia.

2.6 DIAGNOSES OF MALARIA

Since Charles Laveran first visualized the malaria parasite in blood in 1880 (Sutherland, *et al.,* 2009). The mainstay malaria diagnosis has been microscopic examination of blood.

Fever and septic shock are commonly misdiagnose as severe malaria in Africa, leading to a failure to treat other life threatening illness. In malaria – endemic

areas parasitemia does not ensure a diagnosis of severe malaria because parasitamia can be incidental to other concurrent disease – recent investigation suggest that malaria retinopathy is better (collective sensitivity of 95% and specificity of 90%) than any other clinical or laboratory feature in distinguishing malarial from non-malarial coma (Beare, *et al.*, 2006).

Although blood is the sample most frequently used to make a diagnosis, both saliva and urine have been investigated as alternative, less invasive specimen (Sutherland, *et al.,* 2009).

2.6.1 Symptomatic Diagnosis

Areas that cannot afford even simple laboratory diagnostic test often use only a history of subject fever as the indication to treat for malaria. Using Giemsa-stained blood smears from children in Malawi, a study showed that when clinical predictors (rectal temperature, nail bed pallor and spleenomegaly) were used as treatment indications, rather than using only a history of subjective fevers, a correct diagnosis increased from 21% to 41% of cases, and unnecessary treatment for malaria was significantly decreased (Redds, *et al.,* 2006).

2.6.2 Microscopic Examination of Blood Films

The most economic, preferred, and reliable diagnosis of malaria is microscopic examination blood films because of the four major parasites has distinguishing characteristics. Two sorts of blood film are traditionally used. These films are similar to usual blood films and allow species identification because the parasites appearance is best preserved in this preparation, thick films allow the microscopist to screen a larger volume of blood and are about eleven times more

sensitive than the thin film, so picking up low levels of infection is easier on the thick film; but the appearance of the parasite is much more distorted and therefore distinguishing between the different species can be much more difficult. With the pros and cons of both thick and blood film, an experienced microscopist can detect parasite levels (or parasitemia) down to as low as 0.0000001% of red blood cells. Diagnosis of species can be difficult because the early trophozoites ("ring form") of all the four species look identical and it is never possible to diagnosis species on the basis of a single ring form, species identification is always based on several trophozoites (Warhut , *et al.,* 1996). One important thing to note is that *P. malariae* and *p. knowlesi* (which is the most common cause of malaria in south east Asia look very similar under the microscope. However, *P. knowlesi* parasitaemia increases very fast and cause more severe disease than *P. malariae,* so it's important to identify and treat infections quickly. It is suggested that modern methods such as Polymerace Chain Reaction or monoclonal antibody panels that can distinguish between the two should be used in this part of the world (Mc Cutchan, *et al.,* 2008).

2.6.3 Antigen Tests for Malaria.

For areas were microscopy is not available, or where laboratory staff is not experienced at malaria diagnosis, there are commercial antigen detection test that require only a drop of blood (Pattanasin, *et al.,* 2003) immunochromatographic tests i.e. malaria Rapid diagnostic tests, antigen capture assay or "Dipsticks" been developed, distributed and field tested. These tests use finger stick or venous blood for use in the field. The threshold of the detection by these rapid diagnostic tests is in the range of 100 parasites/ul of blood (commercial kits can range from about 0.002%

2.7 PREVENTION OF MALARIA

Methods used in order to prevent the spread of disease, or to protect individuals in areas where malaria is endemic, include prophylactic drugs, mosquito eradication, and the prevention of mosquito bites. The continued existence of malaria in an area requires a combination of high human population density, high mosquito population density, and high rates of transmission from humans to mosquitoes and from mosquitoes to humans. If any of these is lowered sufficiently, the parasite will sooner or later disappear from that area, as happened in North America, Europe and much of Middle East. However, unless the parasite is eliminated from the whole world, it could become re-established if conditions revert to a combination that favors the parasite's reproduction. Many countries are seeing an increasing number of imported malaria cases due to extensive travel and migration. (Barat, 2006)

Many researchers argue that prevention of malaria may be more cost-effective than treatment of the disease in the long run, but the capital costs required are out of reach of many of the world's poorest people economic adviser Jeffrey Sachs, estimates that malaria can be controlled for US$3 billion in aid per year (Medical News Today, 2007).

The distribution of funding varies among countries. Countries with large populations do not receive the same amount of support. The 34 countries that received a per capita annual support of less than $1 included some of the poorest countries in Africa. Brazil, Eritrea, India, and. Vietnam have, unlike many other developing nations, successfully reduced the malaria burden. Common success factors included conducive country conditions, a targeted technical approach using a package of effective tools, data-driven decision-making, active leadership at all

levels of government, involvement of communities, decentralized implementation and control of finances, skilled technical and managerial capacity at national and sub-national levels, hands-on technical and programmatic support from partner agencies, and sufficient and flexible financing (Barat, 2006).

2.7.1 Prophylactic Drugs for the treatment of malaria.

Several drugs, most of which are also used for treatment of malaria, can be taken preventively. Modern drugs used include mefloquine (Lariam), doxycycline (available generically), and the combination of atovaquone and proguanil hydrochloride (Malarone). Doxycycline and the atovaquone and proguanil combination are the best tolerated with mefloquine associated with higher rates of neurological and psychiatric symptoms. (Jacquerioz, et al., 2009). The choice of which drug to use depends on which drugs the parasites in the area are resistant to, as well as side-effects and other considerations. The prophylactic effect does not begin immediately upon starting taking the drugs, so people temporarily visiting malaria-endemic areas usually begin taking the drugs one to two weeks before arriving and must continue taking them for 4 weeks after leaving (with the exception of atovaquone proguanil that only needs be started 2 days prior and continued for 7 days afterwards). Generally, these drugs are taken daily or weekly, at a lower dose than would be used for treatment of a person who had actually contracted the disease. Use of prophylactic drugs is seldom practical for full-time residents of malaria-endemic areas, and their use is usually restricted to short-term visitors and travelers to malarial regions. This is due to the cost of purchasing the drugs, negative side effects from long-term use, and because some effective anti-malarial drugs are difficult to obtain outside of wealthy nations.

Quinine was used historically, but the development of more effective alternatives such as quinacrine, chloroquine, and primaquine in the 20th century reduced its use. Today, quinine is not generally used for prophylaxis. The use of prophylactic drugs where malaria-bearing mosquitoes are present may encourage the development of partial immunity (Rosenberg, *et al.*, 2009).

2.7.2 Control of Vector for Malaria

Efforts to eradicate malaria by eliminating mosquitoes have been successful in some areas. Malaria was once common in the United States and southern Europe, but vector control programs, in conjunction with the monitoring and treatment of infected humans, eliminated it from those regions. In some areas, the draining of wetland breeding grounds and better sanitation were adequate. Malaria was eliminated from most parts of the USA in the early 20th century by such methods, and the use of the pesticide (CDC, 2004). DDT and other means eliminated it from the remaining pockets in the South by 1951. In 2002, there were 1,059 cases of malaria reported in the US, including eight deaths, but in only five of those cases was the disease contracted in United States

Before DDT, malaria was successfully eradicated or controlled also in several tropical areas by removing or poisoning the breeding grounds of the mosquitoes or the aquatic habitats of the larva stages, for example by filling or applying oil to places with standing water. These methods have seen little application in Africa for more than half a century (Killeen *et al.,* 2002).

Sterile insect technique is emerging as a potential mosquito control method. Progress toward transgenic, or genetically modified, insects suggest that wild mosquito populations could be made malaria-resistant. Researchers at Imperial College London created the world's first transgenic malaria mosquito (Imperial

College London, 2000) with the first plasmodium-resistant species announced by a team at a Case Western Reserve University in Ohio in 2002. Successful replacement of current, populations with a new genetically modified population, relies upon a drive mechanism, such as transposable elements to allow for non.-Mendelian inheritance of the gene of interest. However, this approach contains many difficulties and success is a distant prospect (Ito, *et al.,* 2002). An even more futuristic method of vector control is the idea that lasers could be used to kill flying mosquitoes (Robert, 2009)

2.7.3 Indoor Residual Spraying

Indoor residual spraying (IRS) is the practice of spraying insecticides on the interior walls of homes in malaria affected areas. After feeding, many mosquito species rest on a nearby surface while digesting the blood meal, so if the walls of dwellings have been coated with insecticides, the resting mosquitoes will be killed before they can bite another victim, transferring the malaria parasite.

The first pesticide used for Indoor Residual Spraying was Dihydroxyl Diethyl Tetrachloride (DDT) (CDC, 2004). Although it was initially used exclusively to combat malaria, its use quickly spread to agriculture. In time, pest-control, rather than disease- control, came to dominate DDT use, and this large-scale agricultural use led to the evolution of resistant mosquitoes in many regions. The DDT resistance shown by Anopheles mosquitoes can be compared to antibiotic resistance shown by bacteria. The overuse of anti-bacterial soaps and antibiotics led to antibiotic resistance in bacteria, similar to how over spraying of DDT on crops led to DDT resistance in Anopheles mosquitoes. During the 1960s, awareness of the negative consequences of its indiscriminate use increased, ultimately leading to bans on

agricultural applications of DDT in many countries in the 1970s. Since the use of DDT has been limited or banned for agricultural use for some time, DDT may now be more effective as a method of disease-control.

Although DDT has never been banned for use in malaria control and there are several other insecticides suitable for IRS, some advocates have claimed that bans are responsible for tens of millions of deaths in tropical countries where DDT had once been effective in controlling malaria. Furthermore, most of the problems associated with DDT use is specifically from its industrial-scale application in agriculture, rather than its use in public health (Tia, *et al.,* 2006).

The World Health Organization (WHO) currently advises the use of 12 different insecticides in IRS operations, including DDT as well as alternative insecticides (such as the pyrethroids permethrin and deltamethrin (WHO, 2006). This public health use of small amounts of DDT is permitted under the Stockholm Convention on Persistent Organic Pollutants (POPs), which prohibits the agricultural use of DDT. (Vandenberg, *et al.,* 2009)· However, because of its legacy, many developed countries previously discouraged. DD use even in small quantities (Rosenberg, *et al.,* 2004).

One problem with all forms of indoor residual spraying is insecticide resistance via evolution of mosquitoes. According to a study published on Mosquito Behavior and Vector Control, mosquito species that are affected by IRS are endophilic species (species that tend to rest and live indoors), and due to the irritation caused by spraying, their evolutionary descendants are trending towards becoming exophilic (species that tend to rest and live out of doors), meaning that they are not as affected-if affected at all-by the IRS, rendering it somewhat useless as a defense mechanism (Pates, *et al.,* 2005).

2.7.4 Mosquito Nets and Bed-clothes

Mosquito nets help keep mosquitoes away from people and greatly reduce the infection and transmission of malaria. The nets are not a perfect barrier and they are often treated with an insecticide designed to kill the mosquito before it has time to search for a way past the net. Insecticide-treated nets (ITNs) are estimated to be twice as effective as untreated nets and offer greater than 70% protection compared with no net. (Bachou, 2006). Although ITNs are proven to be very effective against malaria, less than 2% of children in urban areas in Sub-Saharan Africa are protected by ITNs. Since the Anopheles mosquitoes feed at night, the preferred method is to hang a large "bed net" above the center of a bed such that it drapes down and covers the bed completely.

The distribution of mosquito nets impregnated with insecticides such as permethrin or deltamethrin has been shown to be an extremely effective method of malaria prevention, and it is also one of the most cost-effective methods of prevention. These nets can often be obtained for around US $2.50 to US$3.50 (€2 to €3) from the United Nations, the World Health Organization (WHO), and others. ITNs have been shown to be the most cost-effective prevention method against malaria and are part of WHO's Millennium Development Goals (MDGs). While some experts argue that international organizations should distribute ITNs and (long lasting insecticides treated nets) LLINs to people for free in order to maximize coverage (since such a policy would reduce price barriers), others insist that cost-sharing between the international organization and recipients would lead to greater usage of the net (arguing that people will value a net more if they pay for it). Additionally, proponents of cost-sharing argue that such a policy ensures that nets are efficiently allocated to those people who most need them (or are most vulnerable to infection).

Through a "selection effect", they argue, those people who most need the bed nets will choose to purchase them, while those less in need will opt out. However, a randomized controlled trial study of ITNs uptake among pregnant women in Kenya, conducted by economists Pascaline Dupas and Jessica Cohen, found that cost-sharing does not necessarily increase the usage intensity of ITNs, nor does it induce uptake by those most vulnerable to infection, as compared to a policy of free distribution In some cases, cost-sharing can actually decrease demand for mosquito nets by erecting a price barrier. Dupas and Cohen's findings support the argument that free distribution of ITNs can be more effective than cost-sharing in both increasing coverage and saving lives. In a cost-effectiveness analysis, Dupas and Cohen note that "cost-sharing" is at best marginally more cost effective than free distribution, but free distribution leads to many more lives saved (Cohen, *et al.,* 2010)

The researchers base their conclusions about the cost-effectiveness of free distribution on the proven spillover benefits of increased ITN usage (Hawley, *et al.,* 2003). When a large number of nets are distributed in one residential area, their chemical additives help reduce the number of mosquitoes in the environment. With fewer mosquitoes .in the environment, the chances of malaria infection for recipients and non-recipients are significantly reduced. (In other words, the Importance of the physical barrier effect of ITNs decreases relative to the positive externality effect of the nets in creating a mosquito-free environment when ITNs are highly concentrated in one residential cluster or community).

For maximum effectiveness, the nets should be re-impregnated with insecticide every six months. This process poses a significant logistical problem in rural areas. New technologies like Olyset or DawaPlus allow for production of long-lasting insecticidal mosquito nets (LLINs), which release insecticide for

approximately 5 years and cost about us & 5.50 ITNs. ITNs protect people sleeping under the net and simultaneously kill mosquitoes that contact the net. Some protection is also provided to others by this method, including people sleeping in the same room but not under the net.

While distributing mosquito nets is a major component of malaria prevention, community education and awareness on the dangers of malaria are associated with distribution campaigns to make sure people who receive a net know how to use it. "Hang Up" campaigns, such as the ones conducted by volunteers of the International Red Cross and Red Crescent Movement consist of visiting households that received a net at the end of the campaign or just before the rainy season, ensuring that the net is being used properly and that the people most vulnerable to malaria, such as young children and the elderly, sleep under it. A study conducted by the CDC in Sierra Leone showed a 22 percent increase in net utilization following a personal visit from a volunteer living in the same community promoting net usage. A study in Togo showed similar improvements (IFRCRC, 2009)

Mosquito nets are often unaffordable to people in developing countries, especially for those most at risk. Only 1 out of 20 people in Africa own a bed net. Nets are also often distributed though vaccine campaigns using voucher subsidies, such as the measles campaign for children. A study among Afghan refugees in Pakistan found that treating top-sheets and chaddars (head coverings) with permethrin has similar effectiveness to using a treated net, but is much cheaper. (Rowland, *et al.,*1999).

Another alternative approach uses spores of the fungus *Beauveria bassiana,* sprayed on walls and bed nets, to kill mosquitoes. While some mosquitoes have

developed resistance to chemicals, they have not been found to develop a resistance to fungal infections. (Rowland, *et al.,* 1999).

A research carried out by Onwujekw, *et al;* 2004 on how to acquire mosquito bed net in Nigerian found out that access to mosquito net was limited; and only a few outlets carry them and consumers would have to travel fairly far to them. About 92% of respondents said that the closest place they could purchase a net was an outdoor market, and that they average time to get there would be approximately 1 hour by bus. Other sources also revealed that non-governmental organizations (NGO's) such as world Health Organization (WHO), Planned Parentalhood Federation of Nigeria (PPFN), Society for Family Health (SFH), Global HIV/AIDS Initiative in Nigeria (GHAIN) etc from time to time give out mosquito bed net freely most especially to nursing mothers and pregnant women in hospitals, house to house or wards.

2.7.5 Vaccination

Immunity (or, more accurately, tolerance) does occur naturally, but only in response to repeated infection with multiple strains of malaria (Farnert, *et al.,* 2009).

Vaccines for malaria are under development, with no completely effective vaccine yet available. The first promising studies demonstrating the potential for a malaria vaccine were performed in 1967 by immunizing mice with live, radiation-attenuated sporozoites, providing protection to about 60% of the mice upon subsequent injection with normal, viable sporozoites (Nussenweig, *et al,* 1967). Since the 1970s, there has been a considerable effort to develop similar vaccination strategies within humans. It was determined that an individual can be protected from a *P. falciparum* infection if they receive over 1,000 bites from infected, irradiated mosquitoes (Hoffman, *et al.,* 2002)

It has been generally accepted that it is impractical to provide at-risk individuals with this vaccination strategy, but that has been recently challenged with work being done by Dr. Stephen Hoffman, one of the key researchers who originally sequenced the genome of Plasmodium *falciparum*. His work most recently has revolved around solving the logistical problem of isolating and preparing the parasites equivalent to 1000 irradiated mosquitoes for mass storage and inoculation of human beings. The company has recently received several multi-million dollar grants from the Bill & Melinda Gates Foundation and the U.S. government to begin early clinical studies in 2007 and 2008. The Seattle Biomedical Research Institute (SBRI), funded by the Malaria Vaccine Initiative, assures potential volunteers that "the 2009 clinical trials won't be a life-threatening experience. While many volunteers [in Seattle] will actually contract malaria, the cloned strain used in the experiments can be quickly cured, and does not cause a recurring form of the disease. Some participants will get experimental drugs or vaccines, while others will get placebo (Doughton, *et al.,* 2008).

Instead, much has been performed to try and understand the immunological processes that provide protection after immunization with irradiated sporozoites. After the mouse vaccination study in 1967 (Nussenzweig, *et al.,* 1967). It was hypothesized that the injected sporozoites themselves were being recognized by the immune system, which was in turn creating antibodies against the parasite. It was determined that the immune system was creating antibodies against the circumsporozoite protein (CSP) which coated the sporozoite (Zavala, *et al.,* 1983). Moreover, antibodies against CSP prevented the sporozoite from invading hepatocytes (Hollingdale, 1984). CSP was therefore chosen as the most promising protein on which to develop a vaccine against the malaria sporozoite. It is for these

historical reasons that vaccines based on CSP are the most numerous of all malaria vaccines.

Presently, there is a huge variety of vaccine candidates on the table. Pre-erythrocytic vaccines (vaccines that target the parasite before it reaches the blood), in particular vaccines based on CSP, make up the largest group of research for the malaria vaccine. There have been recent breakthroughs in vaccines that seek to avoid more severe pathologies of malaria by preventing adherence of the parasite to blood venules and placenta, but financing is not yet in place for trails (Andersen, *et al.,* 2010). Other potential vaccines include those that seek to induce immunity to the blood stages of the infection and transmission-blocking vaccines that would stop the development of the parasite in the mosquito right after the mosquito has taken a bloodmeal from an infected person (Matuschewsk, 2006). It is hoped that the knowledge of the P. *falciparum* genome, the sequencing of which was completed in 2002, (Gardner, *et al.,* 2002) will provide targets for new drugs or vaccines.

The first vaccine developed that has undergone field trials, is the SPf66, developed by Manuel Elkin Patarrovo in 1987. It presents a combination of antigens from the sporozoite (using CSP repeats) and merozoite parasites. During phase I trials a 75% efficacy rate was demonstrated and the vaccine appeared to be well tolerated by subjects and immunogenic. The phase IIb and III trials were less promising, with the efficacy falling to between 38.8% and 60.2%. A trial was carried out in Tanzania in 1993 demonstrating the efficacy to be 31 % after a years follow up, however the most recent (though controversial) study in The Gambia did not show any effect. Despite the relatively long trial periods and the number of studies carried out, it is still not known how the SPf66 vaccine confers immunity; it therefore remains an unlikely solution to malaria. The CSP was the next vaccine developed

that initially appeared promising enough to undergo trials. It is also based on the circumsporoziote protein, but additionally has the recombinant (Asn-Ala-Prol5Asn-Val-Asp-Pro)2-Leu-Arg(R32LR) protein covalently bound to a purified Pseudomonas aeruginosa toxin (A9). However at an early stage a complete lack of protective immunity was demonstrated in those inoculated. The study group used in Kenya had an 82% incidence of parasitaemia whilst the control group only had an 89% incidence. The vaccine intended to cause an increased T-lymphocyte response in those exposed, this was also not observed.

The efficacy of Patarroyo's vaccine has been disputed with some US scientists concluding in The Lancet (1997) that "the vaccine was not effective and should be dropped" while the Colombian accused them of "arrogance" putting down their assertions to the fact that he came from a developing country.

The RTS, S/AS02A vaccine is the candidate furthest along in vaccine trials. It is being developed by a partnership between the PATH Malaria Vaccine Initiative (a grantee of the Gates Foundation), the pharmaceutical company, GlaxoSmithKline, and the Walter Reed Army Institute of Research (Heppner DG, *et al.,* 2005).In the vaccine, a portion of CSP has been fused to the immunogenic antigen" of the hepatitis B virus; this recombinant protein is injected alongside the potent AS02A adjuvant (Matuschewski, 2006). In October 2004, the RTS,S/AS02A researchers announced results of a Phase IIb trial, indicating the vaccine reduced infection risk by approximately 30% and severity of infection by over 50%. The study looked at over 2,000 Mozambican children (Alonso, *et al.,* 2004). More recent testing of the RTS, S/AS02A vaccine has focused on the safety and efficacy of administering it earlier in infancy: In October 2007, the researchers announced results of a phase conducted on 214 Mozambican infants between the ages of 10 and 18 months in

which the full three-dose course of the vaccine led to a 62% reduction of infection with no serious side effects save some pain at the point of injection (Aponte, *et al.*, 2007). Further research will delay this vaccine from commercial release until around 2011 (Aponte, *et al.*, 2007)

On 6 April 2010, Crucell, a Dutch biopharmaceutical company, has signed a binding letter of agreement with GlaxoSmithKline Biologicals (GSK) to collaborate on developing malaria vaccine candidate.

Malaria vaccine strategies can be categorized by their intended primary mode of protection and the stage of the parasite lifecycle which they target. The mode of action of the vaccine candidate will determine the type of trial that is used to evaluate their efficacy.

PRE-ERYTHROCYTIC

Pre-erythrocytic vaccines are designed to prevent the establishment of the liver-stage of the malaria infection (and thus the subsequent release of primary merozoites into the blood) by targeting either the blood-borne sporozoites or the infected hepatocytes. These types of vaccines can be evaluated in challenge studies during which volunteers with no history of previous malaria infection are immunized and then challenged with sporozoites, along with their unimmunized counterparts. It is currently not possible to directly measure the number of sporozoites which establish in the liver, nor is it possible to quantify the number of primary merozoites released; therefore, the appearance of the blood-stage infection must serve as a proxy. In the case of a perfect vaccine, the blood-stage infection should be completely absent, somewhat simplifying the trial. This is seldom if ever the case, however. Instead, a delay in the appearance of a blood-stage infection in vaccinated

individuals is interpreted to mean that fewer sporozoites were able to invade and differentiate in the liver. Experimental evidence supports this as the mode of action of at least two liver stage vaccines, FP9 and RTS,S, rather than reduced growth rate in the blood or increased duration of the liver stage (Bejon, et al; 2005). Vaccine efficacy is defined as one minus the ratio of the individuals who remain parasite-free for a specified period of time (usually 21-60 days) in the vaccinated (N_v) over the unvaccinated group (N_{uv}) (Kester *et al;* 2003). In the event that some of the vaccinees are not completely protected, the average time to detection of the infection (delayed patency) in the vaccinated group is compared to that of the unvaccinated group (Webster *et al;* 2005). This may become the primary endpoint if most (or all) of the vaccinees develop blood-stage infections.

If challenge studies are successful, it is then necessary to evaluate the safety and efficacy of the vaccine under conditions of natural inoculation in individuals with some existing immunity or exposure to the parasite in other words, in situations and populations where the vaccine may be deployed if it becomes licensed. Because the exact time of inoculation, or infectious mosquito bite, is not known, days to blood-stage patency cannot be used as a measure of vaccine efficacy. Instead, volunteers may be treated to ensure that any existing malaria parasites are completely cleared from both the blood and the liver. They are vaccinated and then followed by both active and passive case detection along with a control cohort (Bojang, et al; 2001). The time to the first parasitemia in each individual is recorded and the groups are compared by survival analysis, typically by calculating the hazards ratio using Cox regression. Vaccine efficacy is defined as one minus the hazards ratio. The time to the first clinical episode can be evaluated similarly by replacing active surveillance

with passive detection. A significant delay in the time to first clinical episode and vaccine efficacy can be calculated. (Alonso, et al; 2004)

BLOOD-STAGE

Blood-stage vaccines require a different approach than pre-erythrocytic vaccines. Challenge studies are difficult, particularly because the volunteers must be monitored closely and treated at the first microscopic detection of parasites, minimizing the contact between the immune system and the blood-stage infection. In a blood-stage challenge model proposed by (Cheng *et al;* 2007). Individuals were infected by injecting cultured parasites, parasite density was quantified by PCR over several days, and the growth rates were calculated based on the time-series data. The number of parasites was too low to be detectable by microscopy and all volunteers remained asymptomatic until the time curative drug was administered. This model was used to evaluate a 3-part recombinant blood-stage vaccine (Lawrence et al; 2000), but no differences in the growth rate of the parasites in the vaccinated group compared to the control could be measured. This approach has not been validated in other trials.

A perfect, anti-parasitic vaccine would completely prevent blood-stage infection, making the presence or absence of parasites an acceptable measure of vaccine performance. However, in the absence of a perfect vaccine, the ability of the vaccine to reduce parasite load (Genton, et al; 2002) or reduce morbidity from infection (i.e. clinical episodes) can be measured. Clinical episodes of malaria may be defined as any signs or symptoms of malaria accompanied by any parasitemia, or fever accompanied by parasitemia, or fever accompanied by parasitemia in excess of a particular threshold value (D'Alessandro, et al; 1995).

SEXUAL STAGE

Malaria vaccines which target the sexual stage of the parasite, also called transmission-blocking vaccines, are being developed to interrupt the parasite life cycle. In this case, there would be no immediate health benefit to the vaccinated individual. Reduced transmission may lead to reduced morbidity and mortality in the community, but the extent of this effect will depend heavily on what fraction of the total population is immunized. The endpoints which must be used to measure the efficacy of a transmission-blocking vaccine are substantially different than those for the vaccines described above. (Alonso, et al; 1994)

2.7.6 Other Methods of eradication of malaria.

Education in recognizing the symptoms of malaria has reduced the number .of cases in some areas of the developing world by as much as 20%. Recognizing the disease in the early stages can also stop the disease from becoming a killer. Education can also inform people to cover over areas of stagnant, still water e.g. Water Tanks which are ideal breeding grounds for the parasite and mosquito, thus cutting down the risk of the transmission between people. This is most put in practice in urban areas where there are large centers of population in a confined space and transmission would be most likely in these areas. The Malaria Control Project is currently using downtime computing power donated by individual volunteers around the world to simulate models of the health effects and transmission dynamics in order to find the best method or combination of methods for malaria control. This modeling is extremely computer intensive due to the simulations of large human populations with a vast range of parameters related to biological and social factors that influence the spread of the disease. It is expected to take a few months using

2.8 Treatment of malaria.

The treatment of malaria depends on the severity of the disease. Uncomplicated malaria is treated with oral drugs. Whether patients who can take oral drugs have to be admitted depends on the assessment and the experience of the clinician. Severe malaria requires the parenteral administration of anti-malarial drugs. The traditional treatment for severe malaria has been quinine but there is evidence that the artemisinins are also superior for the treatment of severe malaria. A large clinical trial is currently under way to compare the efficacy of quinine and artesunate in the treatment of severe malaria in African children. Active malaria infection with P. falciparum is a medical emergency requiring hospitalization. Infection with P. vivax, P. ovale or P. malariae can often be treated on an outpatient basis. Treatment of malaria involves supportive measures as well as specific antimalarial drugs. Most antimalarial drugs are produced industrially and are sold at pharmacies. However, as the cost of such medicines are often too high for most people in the developing world, some herbal remedies such as Artemisia annua tea have also been developed, and have gained support from international organisations such as *Medecins Sans Frontieres*. When properly treated, someone with malaria can expect a complete recovery (Reeds, *et al.,* 2006).

2.8.1 Counterfeit Anti-malarial Drugs

Sophisticated counterfeits have been found in several Asian countries such as Cambodia, China, (Newton, *et al.,* 2006). Indonesia, Laos, Thailand, Vietnam and are an important cause of avoidable death in those countries WHO have said that studies indicate that up to 40% of artesunate based malaria medications are counterfeit, especially in the Greater Mekong region and have established a rapid

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Reagents and Chemicals

The reagents and chemicals used in this research work were obtained from reputable scientific and chemical companies such as i. Agappe Diagnostics, India, (SGPT, SGOT reagent diagnostic kits), ii. Spectrum (the creative approach to Bioscience) in Egypt; (G-6-PD and LDH reagent diagnostic kits), iii. Randox laboratories Ltd, United Kingdom. (ALP), iv. BDH chemical Ltd, Poole England. (giemsa stain). All reagents and chemicals used were of analytical grade.

3.1.2 Samples

Blood samples were obtained from the haemotology unit of General Hospital Minna and Federal University of Technology, Minna Clinic. The malaria positive samples were used as test while bloods from apparently healthy male and female volunteers with no parasite infections were used as controls. Blood samples were centrifuged, the serum collected, kept in sterile vials and stored frozen until required for analysis. Fresh whole blood was used for G6PD analysis. Heparinised tubes were employed for ALP and LDH but EDTA was used for G6PD, SGOT and SGPT.

3.1.3 Equipments

Some of the equipment used included centrifuging machine (ALC 4217 MKTI) model, water bath (Vamed, England) and spectrophotometer (Spectrum lab 23, Gallen komp England).

3.2 METHODS

3.2.1 Plasmodium Parasite Screening

This was carried out according to the method outlined in Cheesebrough (1998) which involved;

i. Film making – a drop of well mixed (unclotted) blood sample was placed on a clean grease free clean slide, using the edge of another clean slide, the blood was spread to 2-1cm and allow to air dry, by inverting the slide protected from flies.

ii. Parasite Staining – the slides were flooded with Giemsa stain for 10 minutes and diluted with buffer distilled water (pH 7.2) and allowed to react for 45 minutes before washing the buffer distilled water. After drying the microscopy of the slide were undertaken by using an Olympus Model Microscope at a magnification of X100.

3.2.2 Determination of the Heamatocrit

The microhaematocrit method of Green (1976) was used in the determination. An uncalibated capillary tube was filled 2/3 of the volume with blood by capillary action and one end was sealed with crystal seal. The tubes were transferred to the heamatocrit centrifuge and allowed to spin at 12,000rpm for 5 minutes. The packed cell volume was determined using the heamatocrit measuring gauge.

3.2.3 Enzyme Assays

i. Estimation of Serum Glutamate Pyruvate Transminase.

The test principle for the kinetic determination of Alanine Aminotransferase (ALAT) is according to the following reactions.:

L – Alanine + a-ketoglutate $\xrightarrow{ALT}$ pyruvate + L. Glutamate

pyruvate + NADH + H^+ $\xrightarrow{LDH}$ L – Lactate + NAD^+

ALT Alanine Aminotransferase

LDH – Lactate dehydrogenase

It is based on the oxidation of NADH to NAD^+, the resulting decrease in absorbance at 340nm being proportional to the activity of GPT in the sample. This is a modified formulation for the assay of GPT, as recommended by the International Federal of Clinical Chemistry (IFCC). The IFCC reference method include pyrioxal phosphate (PP) which functions as a co-factor in amino acids transamination pp hence increases GPT activity. It avoids falsely low values in sample containing insufficient endogenous pp e.g. patients with myocardial infarction, liver disease and intensive care patients (Beare, *et al.,* 1980).

The laboratory procedure involved taking a working reagent of (1000ul), serum (100ul), which are mixed and incubated at 37°C for 1 minute. The change in absorbance (ΔOD/min) was read at 340nm in a glass cuvette of 1cm path length within 3 minutes.

Calculation

SGPT activity (μ/l) = (ΔOD/min) x 1768

1768 = factor

ii. Estimation of Serum Serum Glutamate OxalateTransaminase (AST)

The test principle is a modified formulation for the assay of GOT as recommended by the IFCC which involves adding pyridoxal phosphate at a level of 0.1mmol/L.

Kinetic determination of Aspartate Aminotransferase (AST) based upon the following reaction.

$$\text{L – Aspartate + a-ketoglutarate} \xrightarrow{\text{AST}} \text{oxaloacetate + L – Glutamate}$$

$$\text{Oxaloacetate + NADH + H}^{+} \xrightarrow{\text{MDH}} \text{L-Malate + NAD}^{+}$$

AST – Aspartate aminotransferase

MDH – Malate dehydrogenase

Nicotinamide Adenine Dinucleotide Hydroxide (NADH) is oxidized to NAD+; the resulting decrease in absorbance at 340nm is directly proportional to the activity of GOT in the sample (Wolf, 1980).

The laboratory procedure involved taking a working reagent of (1000ul), serum (100ul), which are mixed and incubated at 37°C for 1 minute. The change in absorbance (ΔOD/min) was read at 340nm in a glass cuvette of 1cm path length within 3 minutes.

Calculation

SGOT Activity (iu /L) = (ΔOD/min) x 1768

1768 = Factor

Light path 1cm

iii. Estimation of Serum Alkaline Phosphatase

The assay principle is according to the following reaction.

$$\text{Phenylphosphate} \xrightarrow[\text{pH10}]{\text{ALP}} \text{phenol + phosphate}$$

Phenyl liberate is measured in the presence of 4-amino antipyridine and potassium ferricyanide. The presence of sodium arsenate in the reagent stops the enzymatic reaction. The mixture was read at a wave length of 510nm in a cuvette of 1cm light path.

The laboratory method involves setting up tubes for serum sample, serum blank, standard and reagent blank. 2mls of disodium phenyl phosphate carbonate bicarbonate buffer was added to all tubes and incubated for five minutes at 37°C, 50ul of phenol (standard reagent) was added to standard test tube only and 50ul of serum to serum samples, all tubes were incubated for exactly 15 minutes at 37°C. After 15 minutes the blocking reagent (4-Amino antipyridine) was added to all the tubes in a quantity of 0.5ml each, after 0.5ml of sodium arsenate buffer at pH 10 was added in all the tubes, well mixed and 50ul of distilled water was added to serum blank and reagent blank tubes only. They were mixed, allowed to stands for 10 minutes in the dark, and the color intensity measured. This solution was stable for 45 minutes.

Calculation

$$\frac{\text{OD Serum sample} - \text{OD Serum blank}}{\text{OD standard}} \times n$$

n = 142 (1u/L) factor

iv. Determination of Serum G6PD (Kinetic Method)

Test Principle of G6PDH activity in RBC is released by lysing agent present in the reagent. The G6PDH released catalyses the oxidation of glucose 6-phosphate with the reduction of $NADP^+$ to NADPH. The rate of reduction of NADP to NADPH is measured as an increase in absorbance which is proportional to the G6PD activity in the sample.

$$\text{Glu} - 6 - \text{P} + \text{NADP} \xrightarrow{\text{G-6-PDH}} \text{Gluconate} - 6 - \text{P} + \text{NADPH} + \text{H}$$

The laboratory procedure involved pipetting 1ml of G-6-PD working reagent in a cleaned dried test tube with 0.01m of fresh whole blood, mixed well and incubated for 5-10 minute at room temperature (26°C) after which 2.0ml of starter reagent was added. They were mixed again and incubated at 37°C for 5 minutes. The change in absorbance was read after 1,2 and 3 minutes at a wavelength of 340nm.

Calculation:

$$\text{G6PDH Activity} = \frac{\Delta A \times 4778}{\text{Hb (g/dl)}}$$

$$\text{Hb calculation} = \frac{\text{PCV}}{3}$$

v. Estimation of Lactate Dehydrogenase

The quantitative, diagnostic determination of LDH kinetic ultraviolet method follows the principle below.

LDH catalyzes the reaction between pyruvate and NADH to produce NAD^+ and L – lactate.

$$\text{Pyruvate} + \text{NADH} + H^+ \xrightarrow{\text{LDH}} \text{L-Lactate} + NAD^+$$

The initial rate of the NADH oxidation is directly proportional to the catalytic LDH activity. It was determined by measuring the decrease in absorbance at 340nm.

The laboratory procedure involved pipetting into a cuvette at 37°C at a working reagent (1ml) and sample of (20ul), which are mixed. The initial absorbance was read after 30 seconds and was read again after 1, 2 and 3 minutes. The mean absorbance change per minute (ΔA/min) was determined.

Calculation:

8095 x ΔA 340nm/min

8095 = factor.

In order to obtain reproducible results. A number of precautions were taken. These included; caution against all interferences which may cause a false positive or negative result; All the sample vials used were well cleaned to prevent any contamination. All reagents and chemicals used were authentic and properly stored and the guideline was strictly followed.

CHAPTER FOUR

4.0 RESULTS

4.1 Serum Enzyme Activity in Malaria Patients and Control

SGPT, SGOT, ALP, LDH and G-6-PD and two haematological parameters (PCV and Hb), were analysed in malaria and control subjects. The enzyme activities is presented in a tabular form while that of PCV and Hb is represented as graphs.

Most of the results showed an increase in the enzyme activity of subjects with malaria parasite infection.

Table 4.1 below shows the different enzyme activities of test and control subjects. There was a significant increase ($P<0.05$) in the mean level of SGPT, SGOT, and ALP for the malaria parasite positive subjects (36.9 ± 2.1; 35.0 ± 2.2; 45.7 ± 1.7,) respectively when compared with the controls (27.5 ± 2.6; 21.9 ± 13; 35.5 ± 1.7,) respectively.G-6-PD of the control subject was significantly ($p<0.05$) higher than the test with means of 0.015 ± 0.08 and 0.012 ± 0.009IU/L respectively. LDH activity was statistically insignificantly different ($P>0.05$) in the test and control subjects, (398.8 ± 27.6 and 329.2 ± 22.4IU/L) respectively.

Table 4.1: Serum Enzyme Activity in malaria patients and control subjects.

	Mean + S.E.M (IU/L)	
Enzymes	Control	Test
SGPT	27.5 ± 2.6	36.9 ± 2.1[a]
SGOT	21.9 ± 1.3	35.0 ± 2.2[a]
ALP	35.5 ± 2.5	45.7 ± 1.7[a]
LDH	329.2 ± 27.6	398.8 ± 22.4[b]
G-6-PD	0.015 + 0.009	0.012 ± 0.008[a]

[a] = $P < 0.05$ [b] = $P > 0.05$

[a] = $P < 0.05$ is just to outline a level of significant neither or not to use a value of statistical analysis.

4.2 Serum Enzyme Activity in Malaria Patients and Control Subjects.

Comparison between male patients and male control subjects represented in table 4.2 shows that there was also a statistically significant increase ($p<0.05$) in mean levels of SGPT, SGOT, and ALP of male test which were (38.4±2.9; 38.9±3.2; 49.6±2.3IU/L) respectively and the male control subjects (27.9±3.8; 21.9±1.8; 33.9±5.1 IU/L) in G-6-PD activity of male, there was a significant decrease (P,).05) in the male test, (0.012±0.010IU/L) compared to the male control (0.016+0.010). LDH also showed an insignificant increase ($P>0.05$) for the LDH activity of male test (312±36.1 IU/L) and male control (304.9±21.3IU/L) when compared.

Table 4.2: Serum enzyme Activity in Male malaria patients and Male Control subject.

	Mean ± SEM (IU/L)	
Enzymes	Male Control	Male Test
SGPT	27.9 ± 3.8	38.4 ± 2.9[a]
SGOT	21.9 ± 1.8	38.9 ± 3.2[a]
ALP	33.9 ± 5.1	49.6 ± 2.3[a]
LDH	312.2 ± 36.1	304.9 ± 21.3[b]
G-6-PD	0.016 + 0.010	0.012 ± 0.010[a]

a = $P < 0.05$ b = $P > 0.05$

[a] = $P < 0.05$ is just to outline a level of significant neither or not to use a value of statistical analysis.

4.3 Serum enzyme Activity in female Malaria Patients and Female Control subject.

When female and female control subjects were compared, a significant ($P<0.05$) increase were observed in the mean levels of the enzymes SGPT, SGOT, LDH and G-6-PD which are (35.6 ± 3.0; 21.2 ± 2.9; 492.7 ± 42.5 and 0.016 ± 0.009IU/L), respectively and female control (27.0 ± 3.6; 21.9 ± 2.0; 344 ± 28.3 and 0.013 ± 0.007IU/L). ALP statistically shows an insignificant ($P>0.05$) in the two subjects.

Table 4.3: Serum enzyme Activity in female Malaria Patients and Female Control subject.

	Mean ± SEM (IU/L)	
Enzymes	Female Control	Female Test
SGPT	27.0 ± 3.6	35.6 ± 3.0[a]
SGOT	21.9 ± 2.0	31.2 ± 2.9[a]
ALP	36.5 ± 2.8	41.9 ± 2.3[b]
LDH	344.0 ± 42.5	492.7 ± 28.3[a]
G-6-PD	0.016 ± 0.009	0.013 + 0.007 [a]

a = P < 0.05 b = P > 0.05

[a] = P < 0.05 is just to outline a level of significant neither or not to use a value of statistical analysis.

4.4 Serum enzyme Activity in Male and Female Malaria Patients.

Table 4.4 is the comparison of the enzyme activity in male and female malaria positive subjects. SGPT, SGOT levels statistically shows an insignificant difference ($P>0.05$) between the two sex while LDH and G-6-PD were significantly ($P<0.05$) increased in the female subjects compared to the male. ALP shows a significantly increased $P<0.05$ in male than female.

Table 4.4: Serum enzyme Activity in Male and Female Malaria Patients.

	Mean ± SEM (IU/L)	
Enzymes	Male Control	Male Test
SGPT	38.9 ± 2.9	35.6 ± 2.9[b]
SGOT	38.9 ± 3.2	31.8 ± 2.9[b]
ALP	49.6 ± 2.3	41.9 ± 2.4[a]
LDH	304.9 ± 21.3	492.7 ± 8.3[a]
G-6-PD	0.012 + 0.010	0.016 ± 0.009[a]

a = $P < 0.05$ b = $P > 0.05$

[a] = $P < 0.05$ is just to outline a level of significant neither or not to use a value of statistical analysis.

HAEMATOLOGICAL RESULTS

Comparison of Hb and PVC generally shows a significant difference $P<0.05$ among the test and control patients, Hb and PCV levels of healthy individuals were generally increased over that of malaria infected patients. These are indicated in figure 4.1, 4.2, 4.3 and 4.4 respectively.

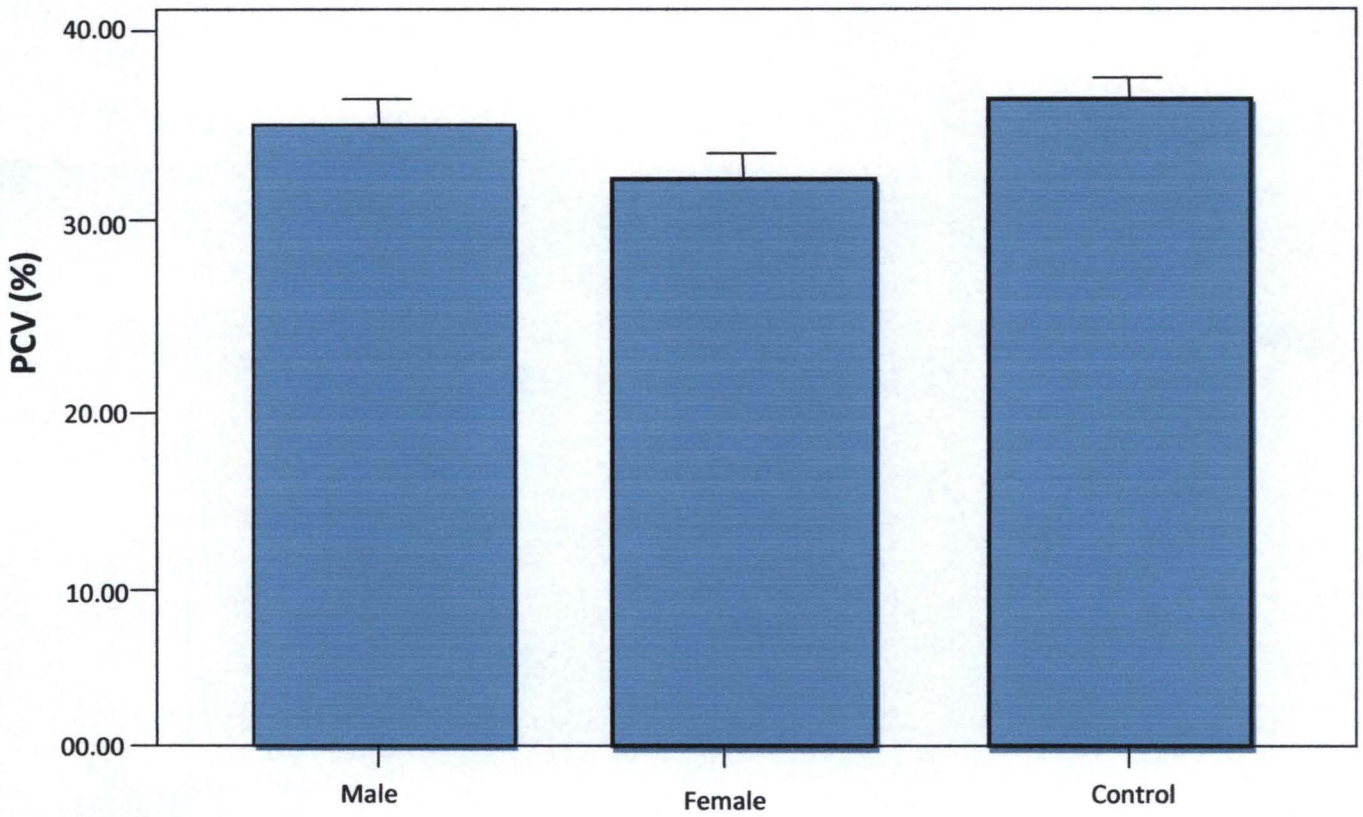


Fig. 4. 1: Packed Cell Volume Levels in Malaria Patients and Controls. There is a significant difference among the test and control group (P<0.05)

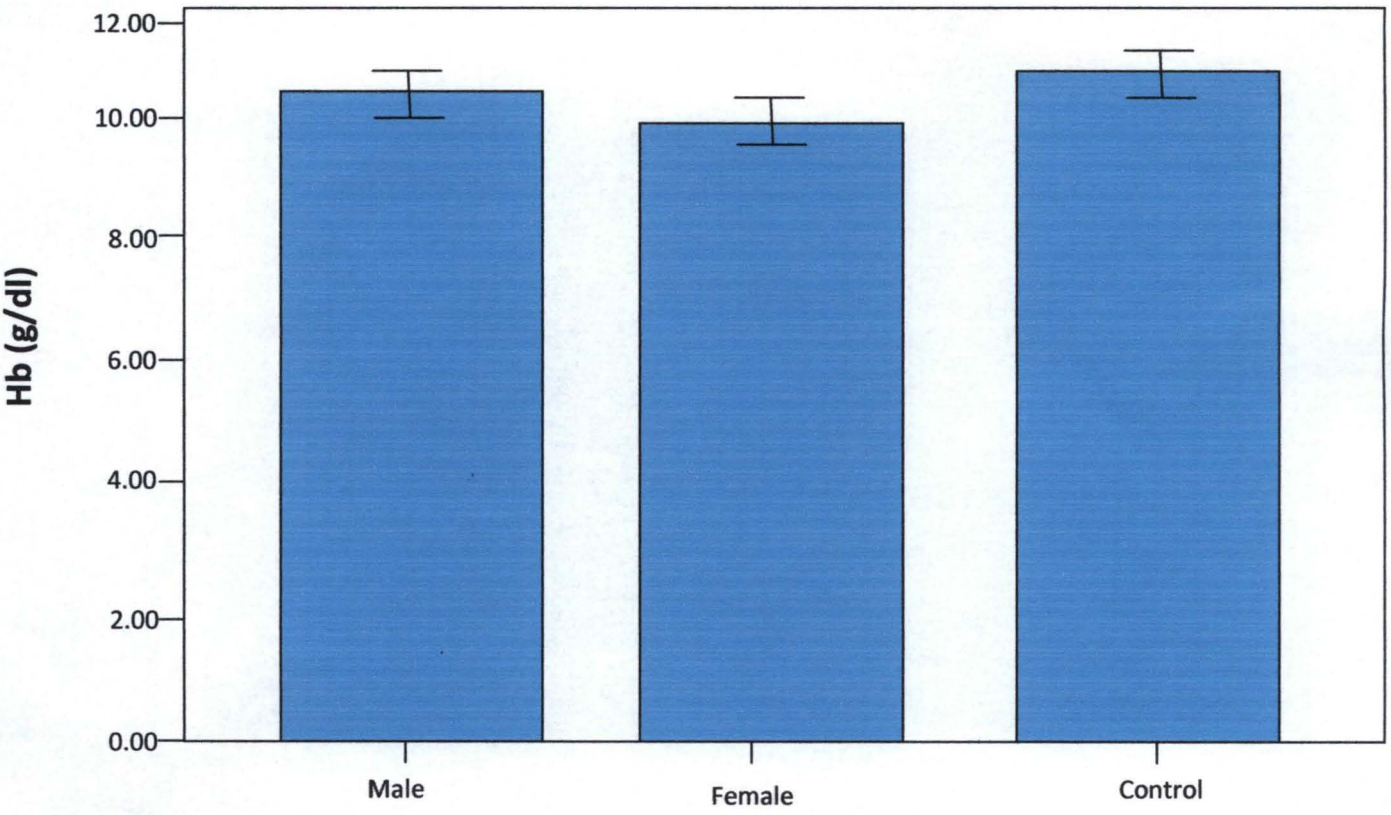


Fig. 4.2: Heamoglobin Levels in Malaria Patients and Controls. There is a significant difference among the test and control group (P<0.05)

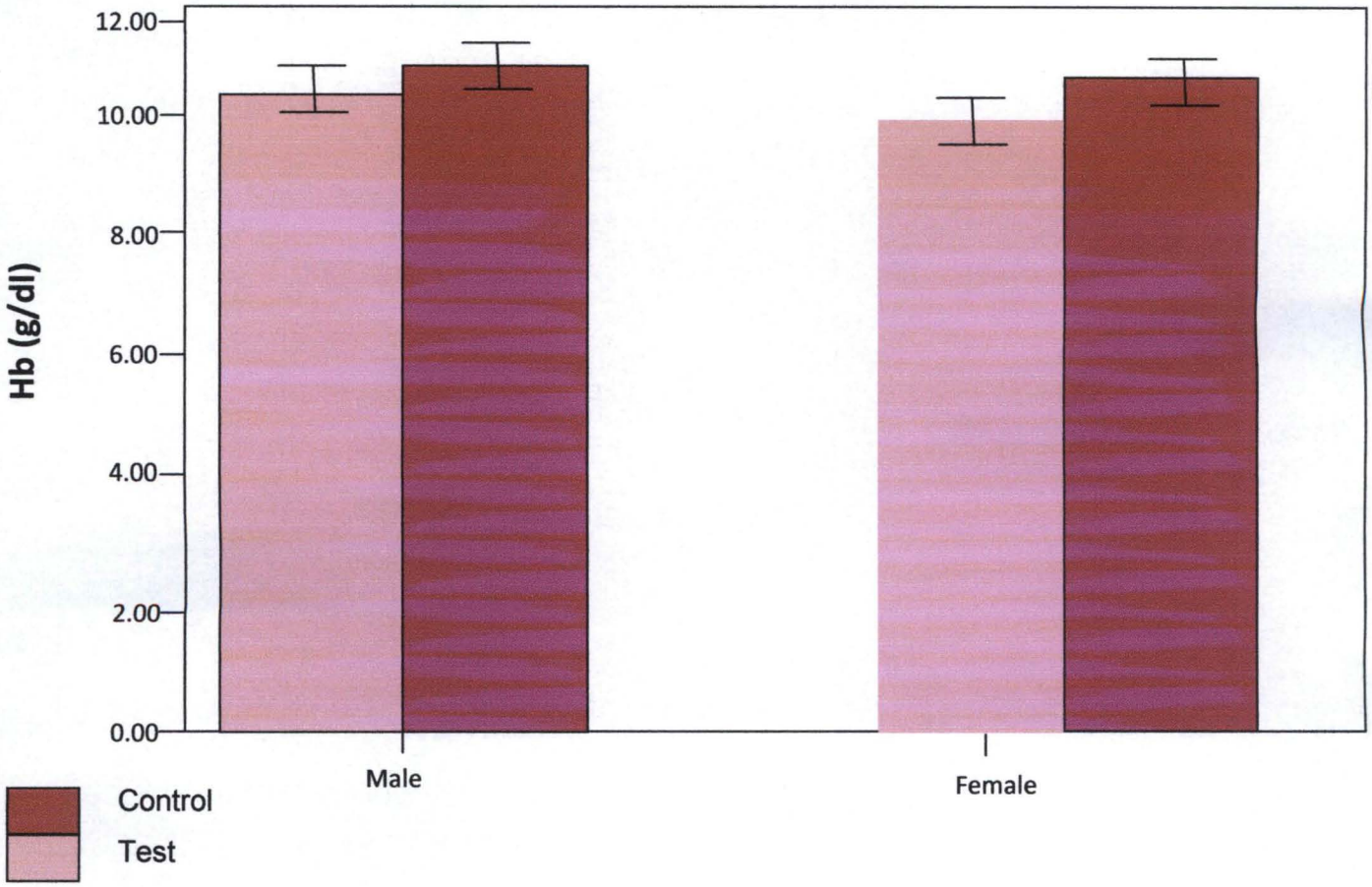


Fig.4.3: Heamoglobin Levels in male and female Patients with their Respective Controls. There is a significant difference among the male and female patients ($P<0.05$)

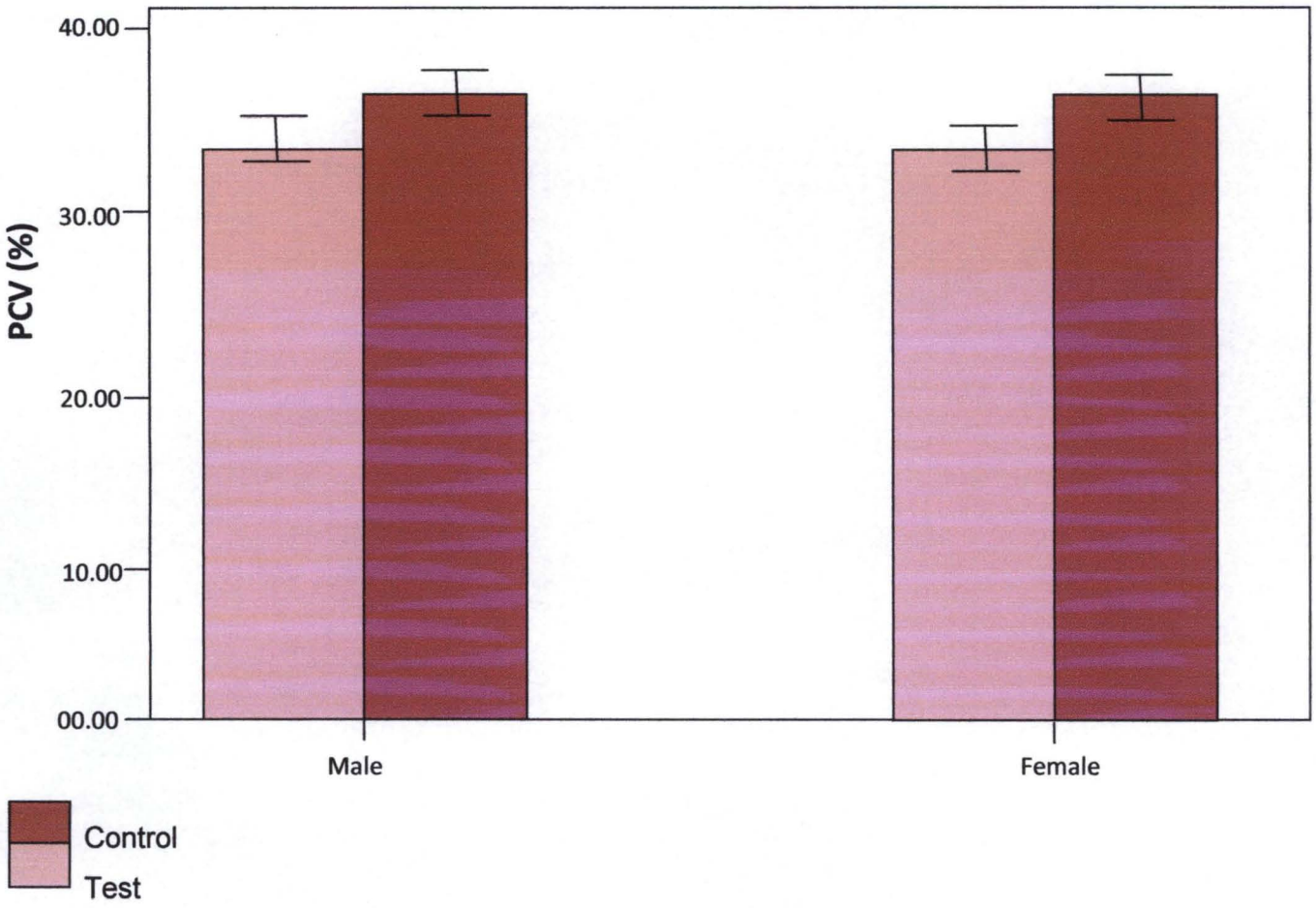


Fig. 4.4: Packed Cell Volume Levels in Male and Female Patients with their Respective Controls. There is a significant difference among the male and female patients (P<0.05)

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSION AND RECOMMENDATION

Investigation on the effect of *plasmodium* parasite infection on Serum enzymes such as serum glutamate oxalate transaminase, serum glutamate pyruvate transaminase, alkaline phosphatase, lactate dehydrogenese and glucose-6-Phosphate Dehydrogenase have become recognized as an important area of research in the pathogenesis of the diseases.

Liver involvement in malaria is common in patients with severe form of the disease and may manifest as Jaundice i.e. raised serum bilirubin, hepatomegaly, elevated liver enzymes like Aspartate and Alanine transaminases. There may be a low and falling serum albumin and prothrombin time way may be prolonged (Amand, *et al.,* 1992).

Malaria caused by *plasmodium falciparum* can cause kidney or liver failure, coma and death. Although infections with other parasites can cause less serious illness, parasites can remain in the liver and cause reappearance of symptoms months or even years later.

Maegraith (1981) postulated that the factors involved in hepatic dysfunction in acute plasmodium falciparum infection involve a synergy between local circulatory failure and centriolobular liver damage and the destruction of the host red blood cells consequent to erythrocytic merogony.

The elevated ALP, SGOT and SGPT activity between the test and control indicates the hepatic stage of the parasites and significant perturbation in the hepatocytic membrane (destruction of the liver parenchyma) leading to their leakage into the blood and general circulation (Muhammed, *et al.,* 2010).

SGOT, SGPT and ALP are used as markers in hepatic diagnosis. In this study, the increase in level of aforementioned enzymes in malaria patients reflects and important in the liver. When the liver is impaired, the liver cells are released into the blood raising the enzymes levels. This is in line with the work of Nyblom *et al.,* 2006 in which the level of the liver enzymes were increased in cirrhosis patients. This is also in consistent with the work of Maduka, *et al.,* (2009) in which the levels of SGOT, SGPT, And ALP were elevated in the work of Adam, *et al.,* (2001) and Price (1997) who suggested that the elevation of the serum enzymes indigenous to the liver is an indication of hepatic dysfunction.

The liver damage may have been caused by free radicals generated by *plasmodium falciparum* in malaria. The deleterious effects were considered to be caused by free radicals produced during peroxide radicals induced by plasmodium falciparum parasites may be responsible for the increase above normal subjects.

However, the different is independent of sex in SGPT, and SGOT, as there is no significant difference between the male and female enzyme activity. The value of the ALP activity of the male is higher than that of the female, this has also been demonstrated in previous investigation. (Ringder, *et al.,* 1979).

Since the LDH is found in clinically significant amount in both the liver and red blood cells, the observed increased in serum LDH activity between the female control and female, as well as the male and female during this study can be pathophsiological process (the combination of Acute hepatocellular injury and red cell heamolysis induced by the invading merozoites). Being a rich source of LDH, the acute liver injury and red blood cell destruction will be followed by a release of LDH into circulation (Garba, *et al.,* 2005). In the course of the study, the LDH activity of the female was significantly ($P<0.05$) higher than that of the male. Higher Ldh activity

in female could be linked to menstruation, which involves cellular destruction. Elevated levels of LDH and changes in the ratio of the LDH isoenzymes usually indicate some type of tissue damage. Usually LDH levels will rise as the cellular destruction begins, peak after some period of time and then begin to fall. Elevated level of LDH may be seen with cerebrovascular accident (CVA, Stroke), drugs such as anesthetics, asprin, narcotics, procainamides and alcohol. LDH can also increase in heamolytic anaemia, pernicious anaemia, (meganoblastic anaemia),infectious mononucleosis, intestinal and pulmonary infarction (tissue death), kidney disease, liver disease, muscular dystrophy, pancreatis, some cancers with some chronic and progressive conditions. It can be concluded that the increase in LDH could be indicative of a stress adaptive response to toxicant, so in the case of the parasite. (Rahman, *et al;* 2002).

Glucose-6-phosphate dehyrogenase enzyme activity of the control is significantly higher than that of the test. The possible reason is as a result of the damage caused on the Red blood cells by the parasite. This is in line with the work of Ruwende *et al;* (1998) who work on G-6PD deficiency in malaria. And suggested that the clinical and pathological manifestations of malaria are consequent upon the release of cellular products and debris from the ruptured erythrocytes and their subsequent phagocytosis by the recticulo - endothelial cells.

The significant difference in hematocrit ad heamoglobin levels signifies anaemia, which is a common manifestation of *falciparum* malaria. Anaemia develops due to profound heamolysis. The degree of anaemia correlates with parasitemia and schizontemia. (Claire, *et al.,* 2004).

Anaemia in malaria is multi-factorial, the causes include obligatory destruction of red blood cells at merogony, accelerated destruction of non-parasitesed red cells

which is a major contributor in severe malaria, bone marrow dysfunction that can persist for weeks, shortened red cell survival and increased spleenic clearance (Claire, *et al.,* 2004).

Erythropoesis is also inhibited leading to reduced levels of hematocrit and hemoglobin. These findings are in agreement with most literature on the pathogenesis of malaria (Dekisi, *et al.,* 2010).

It may be interesting that the G –6 –PD activity Declines with age of RBC, GdB has 62 day half-life for decay of activity, Sustains GSH levels for 100 to 120 day RBC life span, GdA- has normal activity when new, but the activity half-life is only 13 days, Deficiency is due to instability of the enzyme, GdMed has greater instability with 8 day half-life, New cells already have reduced activity, and mature, RBC have enzyme levels < 1% normal activity.

G6PD Hemolysis occurs as a result of Red blood cells will hemolyze or burst when the oxidant stress level becomes too high, Hemolysis occurs in G6PD deficient individuals due to the consumption of certain foods or drugs, Substances that increase the oxidation of glutathione, thereby diminishing the available GSH for oxidation of peroxide, creating a potential for hemolysis, Fava Beans contains vicine and convicine whose metabolites can cause a hemolytic crisis in GdMed individuals, Many anti-malarial drugs, sulfonamides, sulfones and other, drugs produce the same reaction in severely deficient individuals, Can also cause the oxidation of hemoglobin, making it lose the ability to be a reversible oxygen carrier. (Capellini, *et al;* 2008).

Plasmodium protozoans preferentially attack immature RBC but *P. falciparum*can invade RBC of all ages, *Plasmodium* oxidizes RBC NADPH from the Pentose Phosphate pathway for its metabolism, this results in a deficiency of RBC

GSH, most severe in, G6PD deficient individuals, leading to peroxide-induced hemolysis which curtails the development of *Plasmodium* and After several cell cycles the *Plasmodium* can adapt to produce its own G6PD, reducing the adaptive benefit of G6PD deficiency, this could be responsible for its lower activity in malaria patients.

5.2 CONCLUSION

In conclusion, assessment of the enzymes serum Glutamate Oxalate Transaminase, Serum Glutamate pyruvate Transaminase, Alkaline phosphatase, lactate Dehydrogenase and Glucose -6- phosphate Dehydrogenase with the haematological parameter packed cell volume and hemoglobin levels in malaria parasite infected patients could represent and additional and useful parameter in determining the clinical and prognostic aspect of diseases. This is because malaria is a disease whose pathogenesis is not clearly defined, species specific and of Geographical variations.

5.3 RECOMMENDATION

Wrong treatment is sequel to misdiagnosis, since the activities of the enzymes are also increased in some other conditions like hepatitis, Jaundice etc. A person may be misdiagnosed as having hepatitis or jaundice when there is an increase in the activity of these enzymes.

All cases of elevation of SGPT, SGOT, ALP and LDH enzymes should therefore be referred for malaria parasite screening as the increased enzyme activity may be as a result of malaria parasite attack rather than primary liver damage or hepatitis/jaundice.

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APPENDICS

Appendix 1A

T-Test

Group Statistics

	Sample	N	Mean	Std. Deviation	Std. Error Mean
PVC (%)	Test	48	31.2708	5.70457	.82338
	Control	12	34.0000	6.48074	1.87083
Hb (g/dl)	Test	48	10.1958	2.02042	.29162
	Control	12	11.3250	2.16044	.62367

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
PVC (%)	Equal variances assumed	.754	.389	-1.443	58	.154	-2.72917	1.89120	-6.51482	1.05649
	Equal variances not assumed			-1.335	15.538	.201	-2.72917	2.04401	-7.07277	1.61443
Hb (g/dl)	Equal variances assumed	.315	.577	-1.709	58	.093	-1.12917	.66090	-2.45210	.19376
	Equal variances not assumed			-1.640	16.155	.120	-1.12917	.68848	-2.58754	.32920

T-Test

Group Statistics

	Sample	N	Mean	Std. Deviation	Std. Error Mean
SGPT (IU/L)	Test	97	36.93285	20.630389	2.094699
	Control	33	27.52061	14.782331	2.573274

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
SGPT (IU/L)	Equal variances assumed	2.725	.101	2.416	128	.017	9.412239	3.896468	1.702411	17.122068
	Equal variances not assumed			2.837	77.165	.006	9.412239	3.318056	2.805370	16.019108

Appendix 1B

T-Test

Group Statistics

	Sample	N	Mean	Std. Deviation	Std. Error Mean
SGOT (IU/L)	Test	101	35.0495	22.13844	2.20286
	Control	40	21.8906	8.55771	1.35309

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
SGOT (IU/L)	Equal variances assumed	29.312	.000	3.646	139	.000	13.15886	3.60876	6.02370	20.29401
	Equal variances not assumed			5.090	138.970	.000	13.15886	2.58524	8.04738	18.27033

T-Test

Group Statistics

	Sample	N	Mean	Std. Deviation	Std. Error Mean
LDH	Test	46	398.8365	152.07707	22.42254
	Control	15	329.2053	106.90481	27.60270

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
LDH	Equal variances assumed	2.755	.102	1.642	59	.106	69.63119	42.41681	-15.24466	154.50704
	Equal variances not assumed			1.958	33.971	.058	69.63119	35.56233	-2.64245	141.90483

Appendix 1C

T-Test

Group Statistics

	Sample	N	Mean	Std. Deviation	Std. Error Mean
ALP (IU/L)	Test	55	45.7473	12.73544	1.71725
	Control	21	35.5476	11.78591	2.57190

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
ALP (IU/L)	Equal variances assumed	.397	.531	3.185	74	.002	10.19965	3.20285	3.81783	16.58147
	Equal variances not assumed			3.298	38.941	.002	10.19965	3.09250	3.94417	16.45514

T-Test

Group Statistics

	Sex	N	Mean	Std. Deviation	Std. Error Mean
PVC (%)	Male	22	33.1818	5.40402	1.15214
	Female	26	29.6538	5.54215	1.08690
Hb (g/dl)	Male	22	10.7409	2.12797	.45368
	Female	26	9.7346	1.84064	.36098

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
PVC (%)	Equal variances assumed	.466	.498	2.223	46	.031	3.52797	1.58732	.33286	6.72309
	Equal variances not assumed			2.227	45.043	.031	3.52797	1.58392	.33789	6.71806
Hb (g/dl)	Equal variances assumed	.010	.923	1.757	46	.086	1.00629	.57270	-.14650	2.15908
	Equal variances not assumed			1.736	41.900	.090	1.00629	.57977	-.16382	2.17640

Appendix 1D

T-Test

Group Statistics

	Sex	N	Mean	Std. Deviation	Std. Error Mean
SGPT (IU/L)	Male	46	38.39567	19.888744	2.932435
	Female	51	35.61343	21.387533	2.994854

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
SGPT (IU/L)	Equal variances assumed	.220	.640	.661	95	.510	2.782243	4.207323	-5.570349	11.134834
	Equal variances not assumed			.664	94.905	.508	2.782243	4.191459	-5.538962	11.103447

T-Test

Group Statistics

	Sex	N	Mean	Std. Deviation	Std. Error Mean
SGOT (IU/L)	Male	50	38.9219	22.66499	3.20531
	Female	51	31.2530	21.14464	2.96084

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
SGOT (IU/L)	Equal variances assumed	.001	.982	1.759	99	.082	7.66892	4.36053	-.98332	16.32116
	Equal variances not assumed			1.757	98.217	.082	7.66892	4.36356	-.99018	16.32802

Appendix 1E

T-Test

Group Statistics

	Sex	N	Mean	Std. Deviation	Std. Error Mean
LDH	Male	23	304.9352	102.15708	21.30122
	Female	23	492.7378	135.77022	28.31005

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
LDH	Equal variances assumed	1.451	.235	-5.301	44	.000	-187.80261	35.42881	-259.205	-116.401
	Equal variances not assumed			-5.301	40.864	.000	-187.80261	35.42881	-259.360	-116.245

T-Test

Group Statistics

	Sex	N	Mean	Std. Deviation	Std. Error Mean
ALP (IU/L)	Male	27	49.6926	12.00756	2.31086
	Female	28	41.9429	12.44854	2.35255

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
ALP (IU/L)	Equal variances assumed	.004	.952	2.349	53	.023	7.74974	3.29986	1.13105	14.36842
	Equal variances not assumed			2.350	53.000	.023	7.74974	3.29766	1.13547	14.36400

Appendix 1F

T-Test

Group Statistics

	Sample	N	Mean	Std. Deviation	Std. Error Mean
PVC (%)	Male	22	33.1818	5.40402	1.15214
	Control	12	34.0000	6.48074	1.87083
Hb (g/dl)	Male	22	10.7409	2.12797	.45368
	Control	12	11.3250	2.16044	.62367

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
PVC (%)	Equal variances assumed	1.412	.244	-.393	32	.697	-.81818	2.08028	-5.05557	3.41921
	Equal variances not assumed			-.372	19.460	.714	-.81818	2.19714	-5.40951	3.77314
Hb (g/dl)	Equal variances assumed	.405	.529	-.761	32	.452	-.58409	.76769	-2.14782	.97964
	Equal variances not assumed			-.757	22.432	.457	-.58409	.77122	-2.18173	1.01355

T-Test

Group Statistics

	Sample	N	Mean	Std. Deviation	Std. Error Mean
PVC (%)	Female	26	29.6538	5.54215	1.08690
	Control	12	34.0000	6.48074	1.87083
Hb (g/dl)	Female	26	9.7346	1.84064	.36098
	Control	12	11.3250	2.16044	.62367

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
PVC (%)	Equal variances assumed	.594	.446	-2.131	36	.040	-4.34615	2.03984	-8.48313	-.20917
	Equal variances not assumed			-2.009	18.740	.059	-4.34615	2.16365	-8.87898	.18667
Hb (g/dl)	Equal variances assumed	.586	.449	-2.344	36	.025	-1.59038	.67842	-2.96628	-.21448
	Equal variances not assumed			-2.207	18.682	.040	-1.59038	.72060	-3.10036	-.08041

Appendix 1G

T-Test

Group Statistics

	Sample	N	Mean	Std. Deviation	Std. Error Mean
SGPT (IU/L)	Male	46	38.39567	19.888744	2.932435
	Control	33	27.52061	14.782331	2.573274

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
SGPT (IU/L)	Equal variances assumed	1.640	.204	2.657	77	.010	10.875068	4.093507	2.723854	19.026281
	Equal variances not assumed			2.787	76.880	.007	10.875068	3.901399	3.106197	18.643939

T-Test

Group Statistics

	Sample	N	Mean	Std. Deviation	Std. Error Mean
SGPT (IU/L)	Female	51	35.61343	21.387533	2.994854
	Control	33	27.52061	14.782331	2.573274

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
SGPT (IU/L)	Equal variances assumed	2.903	.092	1.898	82	.061	8.092825	4.263476	-.388586	16.574237
	Equal variances not assumed			2.050	81.592	.044	8.092825	3.948529	.237355	15.948296

Appendix 1H

T-Test

Group Statistics

	Sample	N	Mean	Std. Deviation	Std. Error Mean
LDH	Male	23	304.9352	102.15708	21.30122
	Control	15	329.2053	106.90481	27.60270

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
LDH	Equal variances assumed	.049	.825	-.703	36	.487	-24.27012	34.52529	-94.29066	45.75042
	Equal variances not assumed			-.696	29.078	.492	-24.27012	34.86619	-95.57122	47.03099

T-Test

Group Statistics

	Sample	N	Mean	Std. Deviation	Std. Error Mean
LDH	Female	23	492.7378	135.77022	28.31005
	Control	15	329.2053	106.90481	27.60270

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
LDH	Equal variances assumed	.730	.399	3.931	36	.000	163.53249	41.59703	79.16981	247.89518
	Equal variances not assumed			4.136	34.589	.000	163.53249	39.53945	83.22904	243.83595

Appendix 1l

T-Test

Group Statistics

	Sample	N	Mean	Std. Deviation	Std. Error Mean
ALP (IU/L)	Male	27	49.6926	12.00756	2.31086
	Control	21	35.5476	11.78591	2.57190

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
ALP (IU/L)	Equal variances assumed	.104	.748	4.081	46	.000	14.14497	3.46579	7.16869	21.12125
	Equal variances not assumed			4.091	43.513	.000	14.14497	3.45756	7.17452	21.11543

T-Test

Group Statistics

	Sample	N	Mean	Std. Deviation	Std. Error Mean
ALP (IU/L)	Female	28	41.9429	12.44854	2.35255
	Control	21	35.5476	11.78591	2.57190

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
ALP (IU/L)	Equal variances assumed	.134	.716	1.620	47	.075	6.39524	3.51346	-.67283	13.46341
	Equal variances not assumed			1.835	44.430	.073	6.39524	3.48556	-.62754	13.41801

Appendix 1J

Group Statistics

	Sample	N	Mean	Std. Deviation	Std. Error Mean
SGPT (IU/L)	Male	46	38.39567	19.888744	2.932435
	Male Control	16	27.99775	15.355557	3.838889

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
SGPT (IU/L)	Equal variances assumed	.709	.403	1.900	60	.062	10.397924	5.473309	-.550324	21.346172
	Equal variances not assumed			2.152	33.779	.039	10.397924	4.830760	.578268	20.217580

T-Test

Group Statistics

	Sample	N	Mean	Std. Deviation	Std. Error Mean
SGPT (IU/L)	Female	51	35.61343	21.387533	2.994854
	Female control	17	27.07153	14.681165	3.560706

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
SGPT (IU/L)	Equal variances assumed	1.916	.171	1.527	66	.131	8.541902	5.592618	-2.624121	19.707925
	Equal variances not assumed			1.836	40.206	.074	8.541902	4.652717	-.860089	17.943893

Appendix 1K

T-Test

Group Statistics

	Sample	N	Mean	Std. Deviation	Std. Error Mean
SGOT (IU/L)	Male	50	38.92188	22.664994	3.205314
	Male Control	20	21.86675	8.316953	1.859727

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
SGOT (IU/L)	Equal variances assumed	13.442	.000	3.266	68	.002	17.055130	5.221556	6.635677	27.474583
	Equal variances not assumed			4.602	67.744	.000	17.055130	3.705756	9.659905	24.450355

T-Test

Group Statistics

	Sample	N	Mean	Std. Deviation	Std. Error Mean
SGOT (IU/L)	Female	51	31.25296	21.144642	2.960842
	Female Control	20	21.91445	9.008353	2.014329

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
SGOT (IU/L)	Equal variances assumed	19.828	.000	1.902	69	.061	9.338511	4.909905	-.456481	19.133503
	Equal variances not assumed			2.608	68.423	.011	9.338511	3.581076	2.193383	16.483638

Appendix 1L

T-Test

Group Statistics

	Sample	N	Mean	Std. Deviation	Std. Error Mean
ALP (IU/L)	Male	27	49.69259	12.007559	2.310856
	Male Control	8	33.92500	14.601932	5.162563

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
ALP (IU/L)	Equal variances assumed	.018	.895	3.108	33	.004	15.767593	5.073024	5.446447	26.088738
	Equal variances not assumed			2.788	9.978	.019	15.767593	5.656157	3.161163	28.374023

T-Test

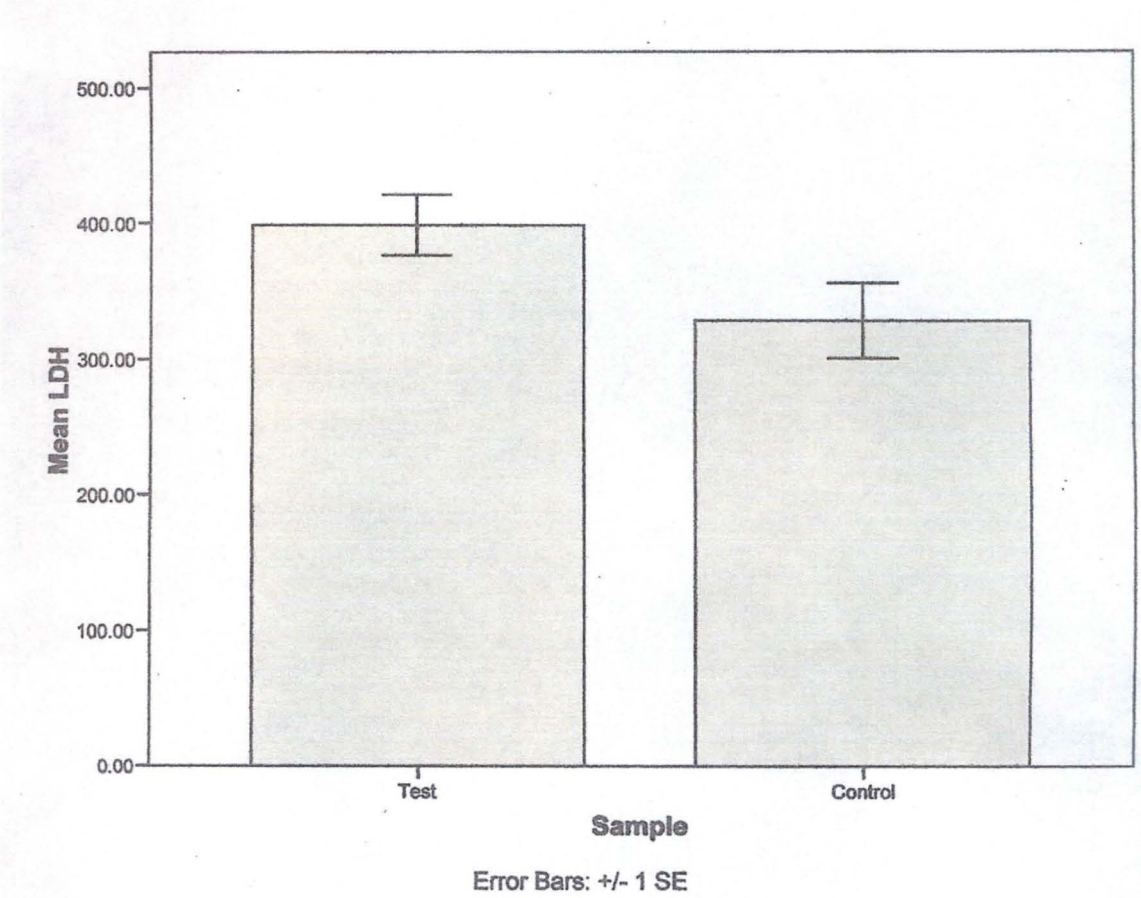
Group Statistics

	Sample	N	Mean	Std. Deviation	Std. Error Mean
ALP (IU/L)	Female	28	41.94286	12.448544	2.352554
	Female Control	13	36.54615	10.212787	2.832518

Independent Samples Test

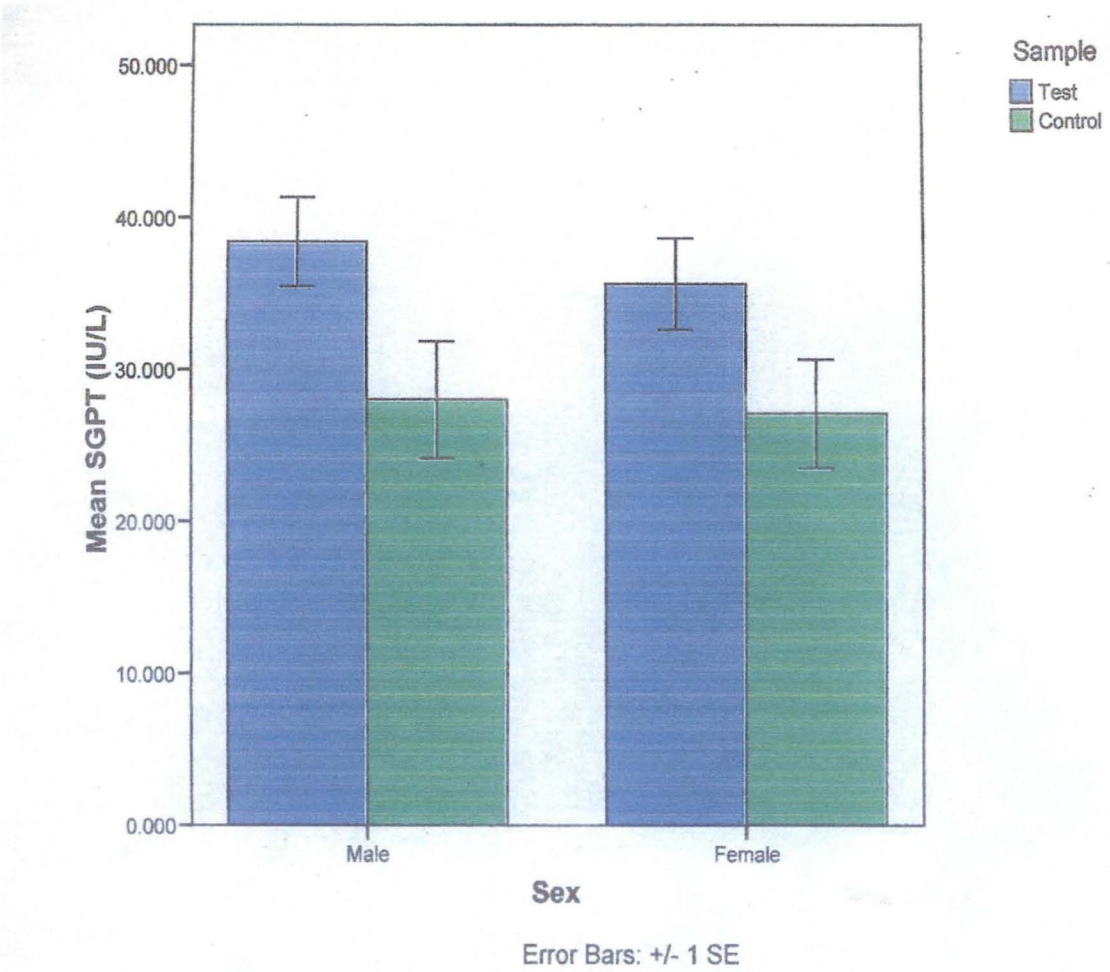
		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
ALP (IU/L)	Equal variances assumed	.314	.578	1.382	39	.181	5.396703	3.902203	-2.617606	13.411015
	Equal variances not assumed			1.466	28.284	.154	5.396703	3.682073	-2.142271	12.935678

Appendix IVE

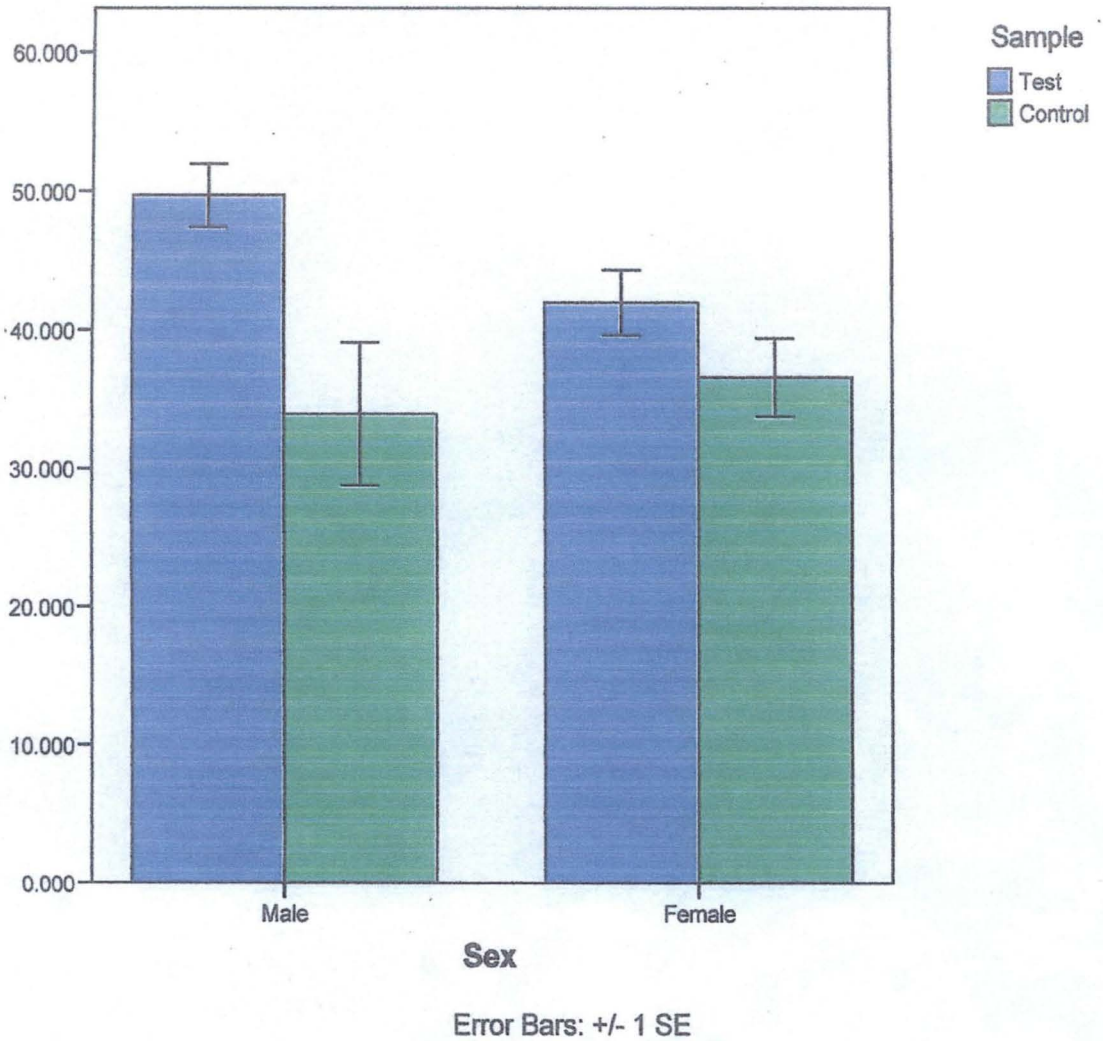


Comparison of mean LDH activity of test and control

Appendix IIA

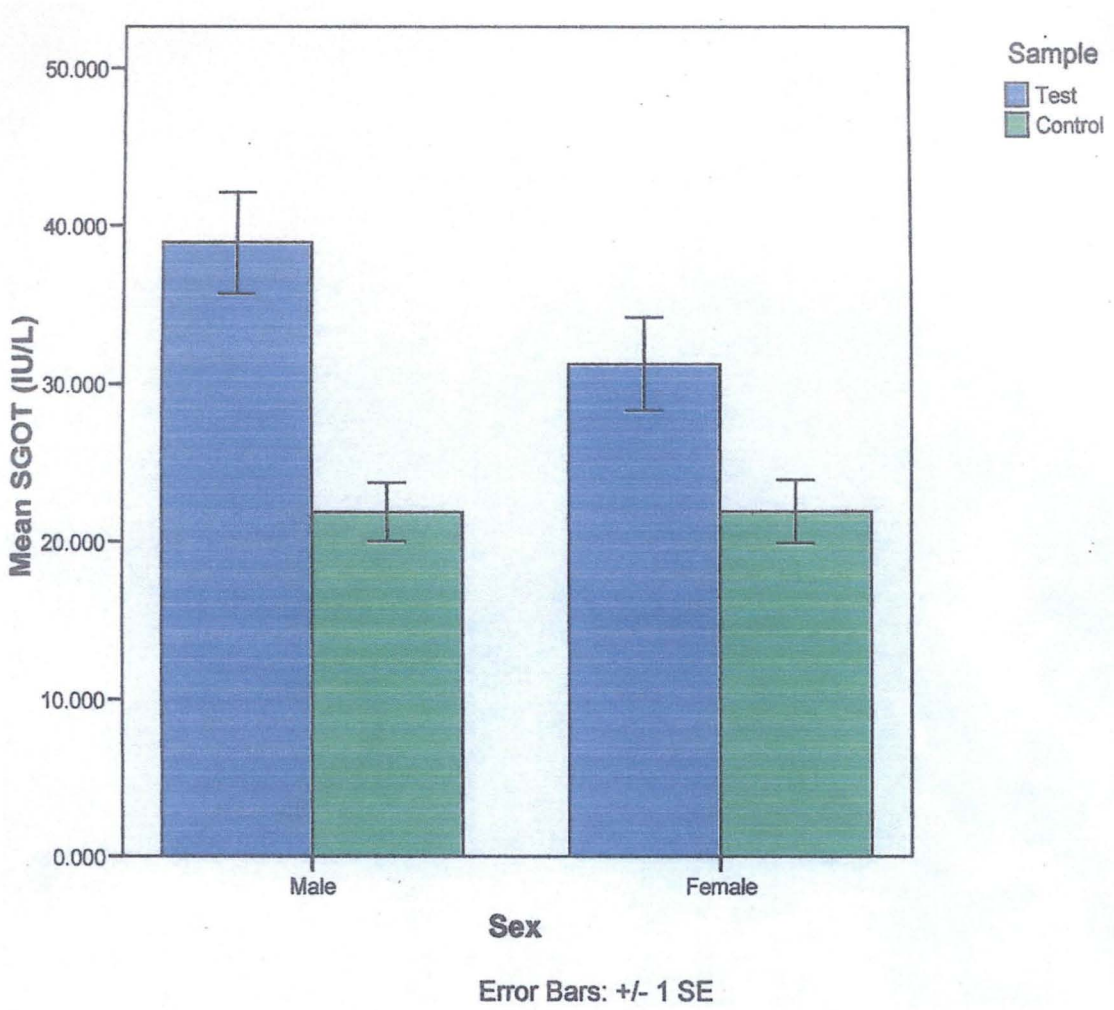


Appendix IIB

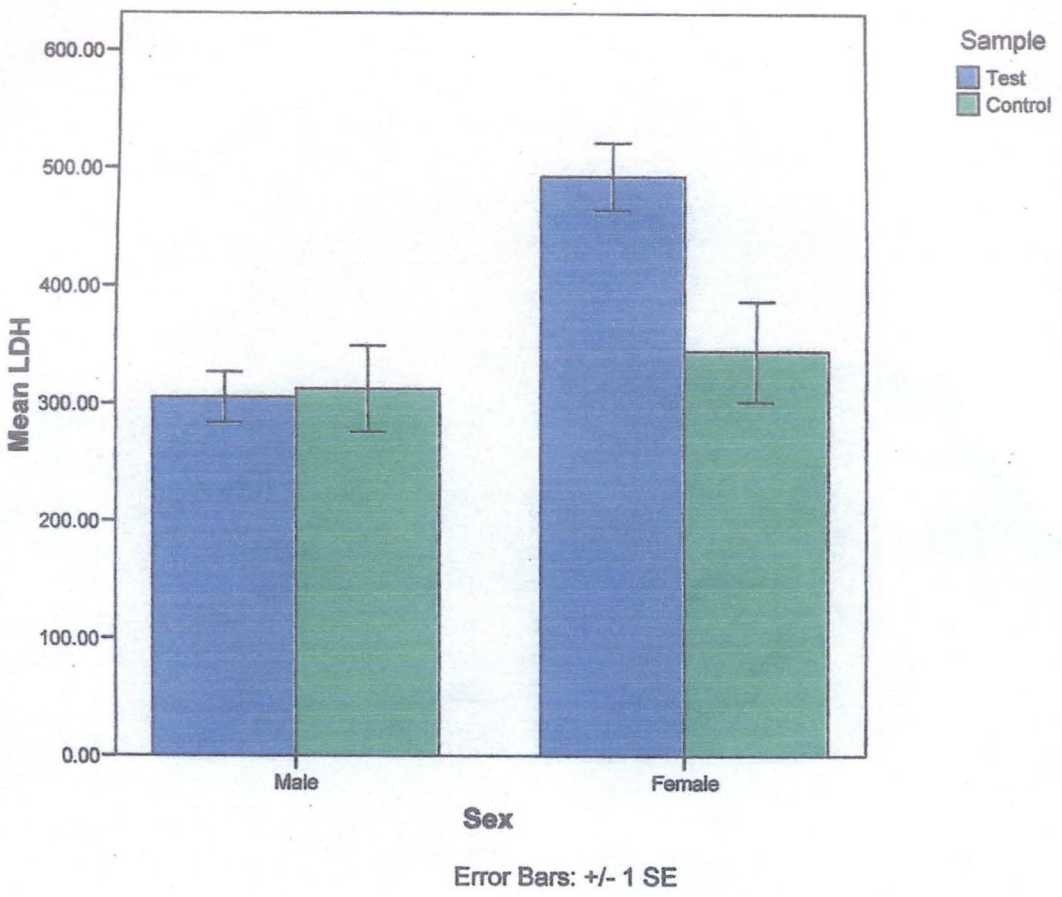


Comparison of mean level of male and female Alp with their respective controls

Appendix IIC

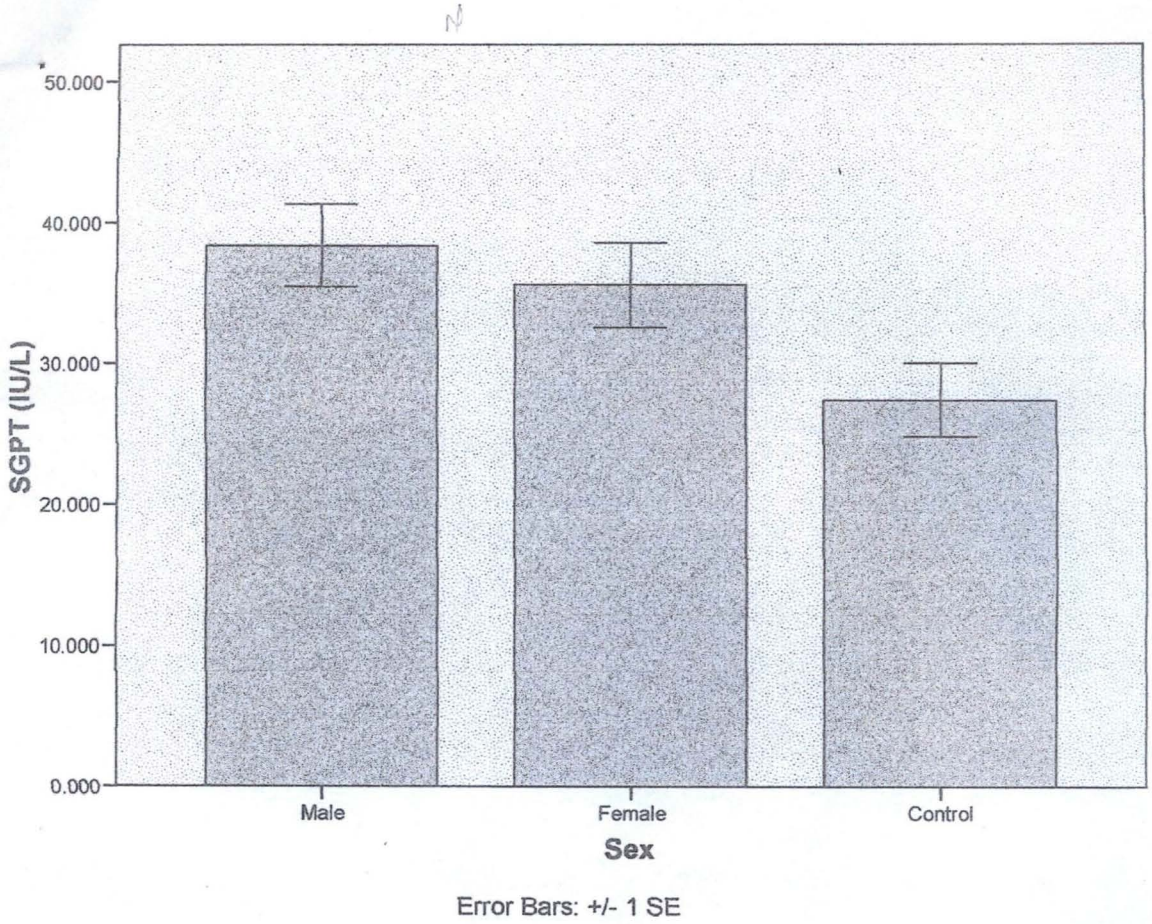


Comparison of mean level of male and female sgot with their respective control



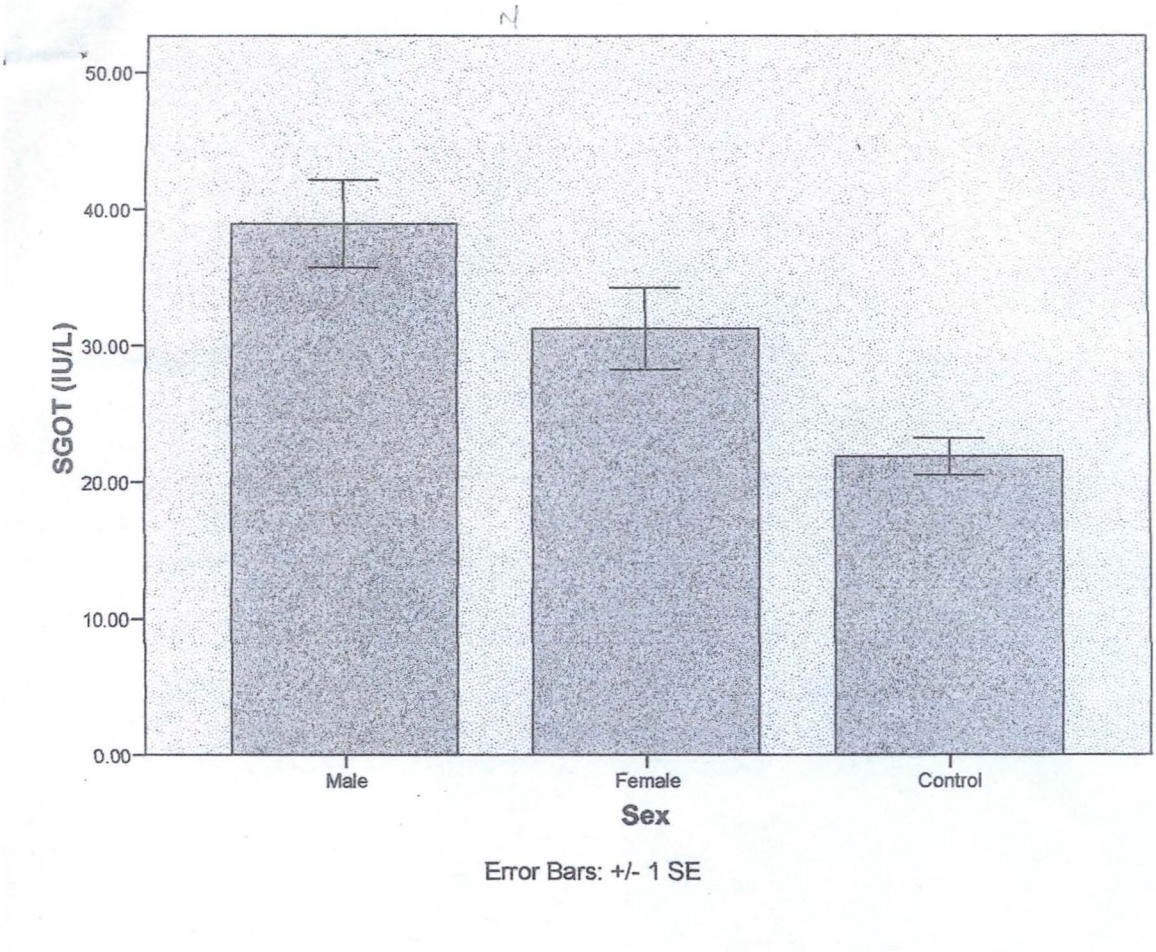
Comparison of mean levels of male and female sgpt with their respective control

Appendix IIIA



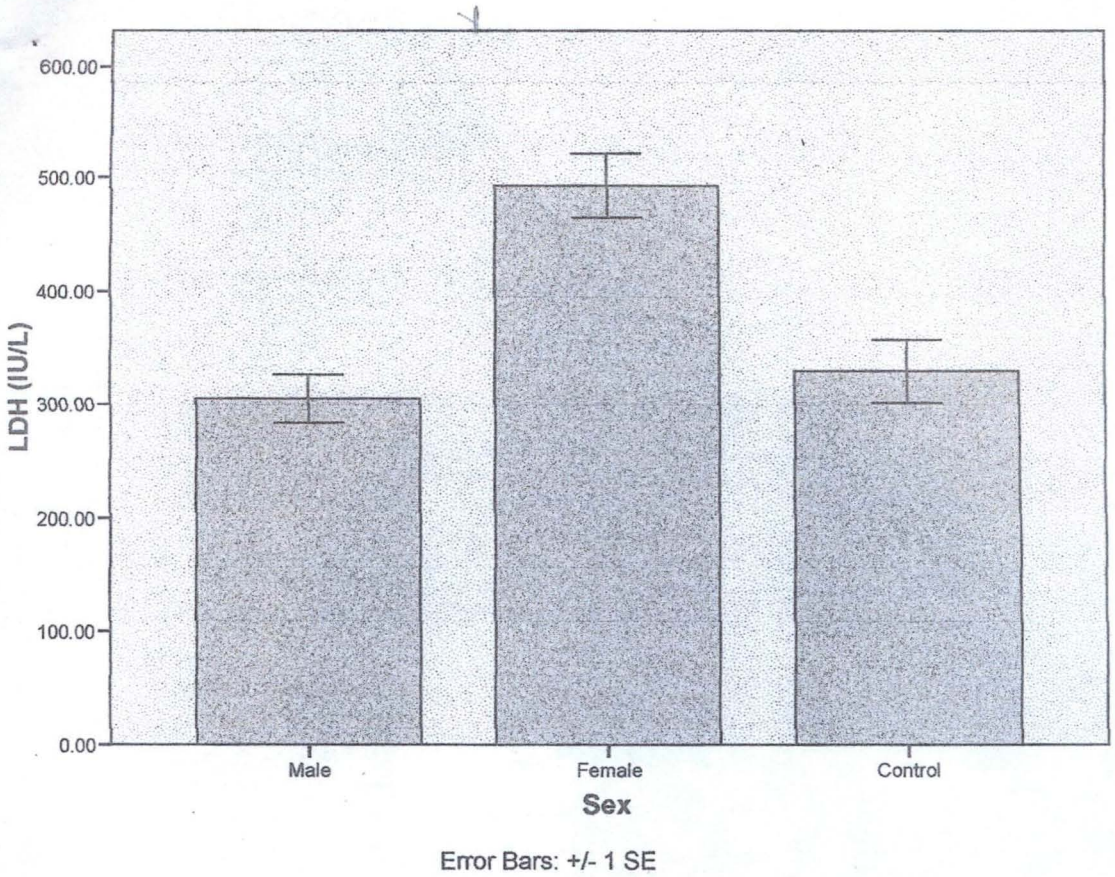
Comparison of mean level of male and female sgpt activity with control

Appendix IIIB



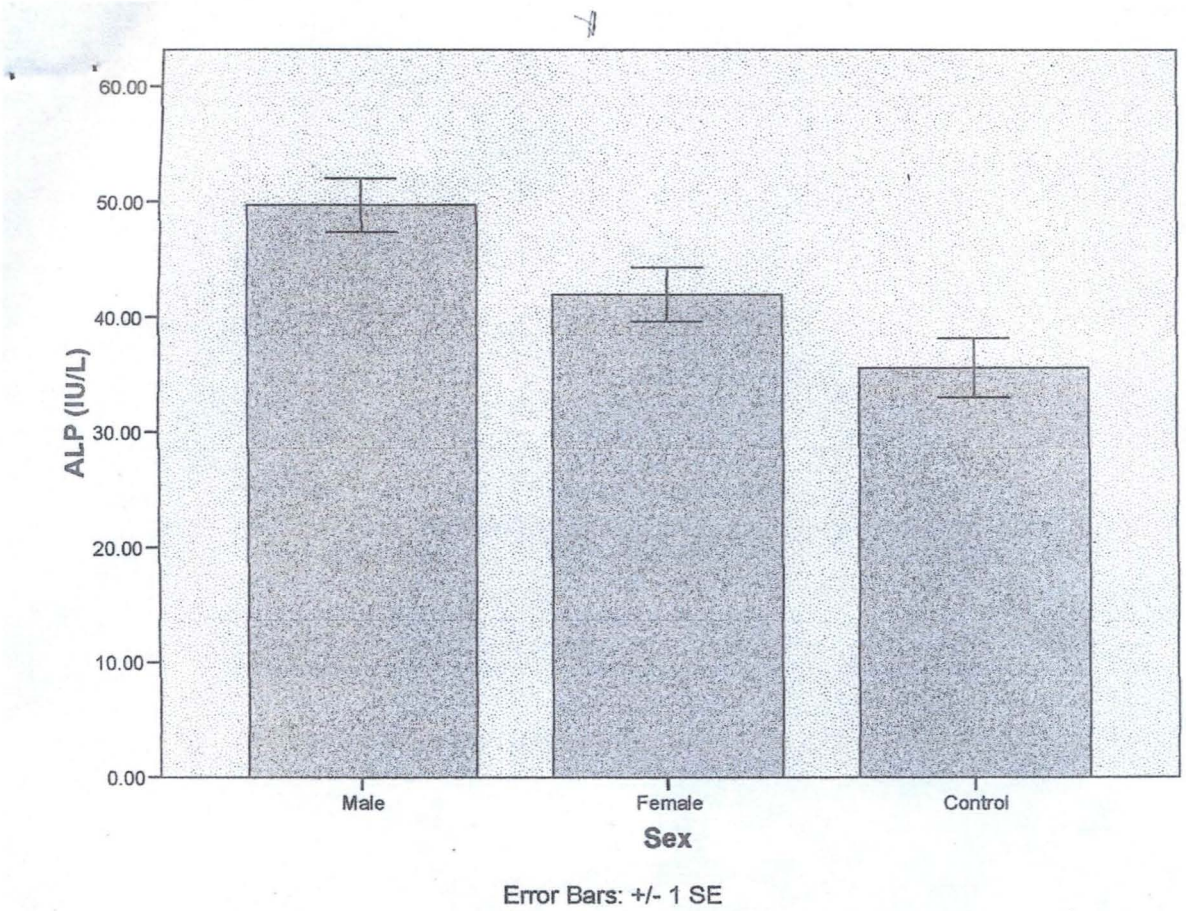
Comparison of mean level of male and female sgpt activity controls

Appendix IIIC



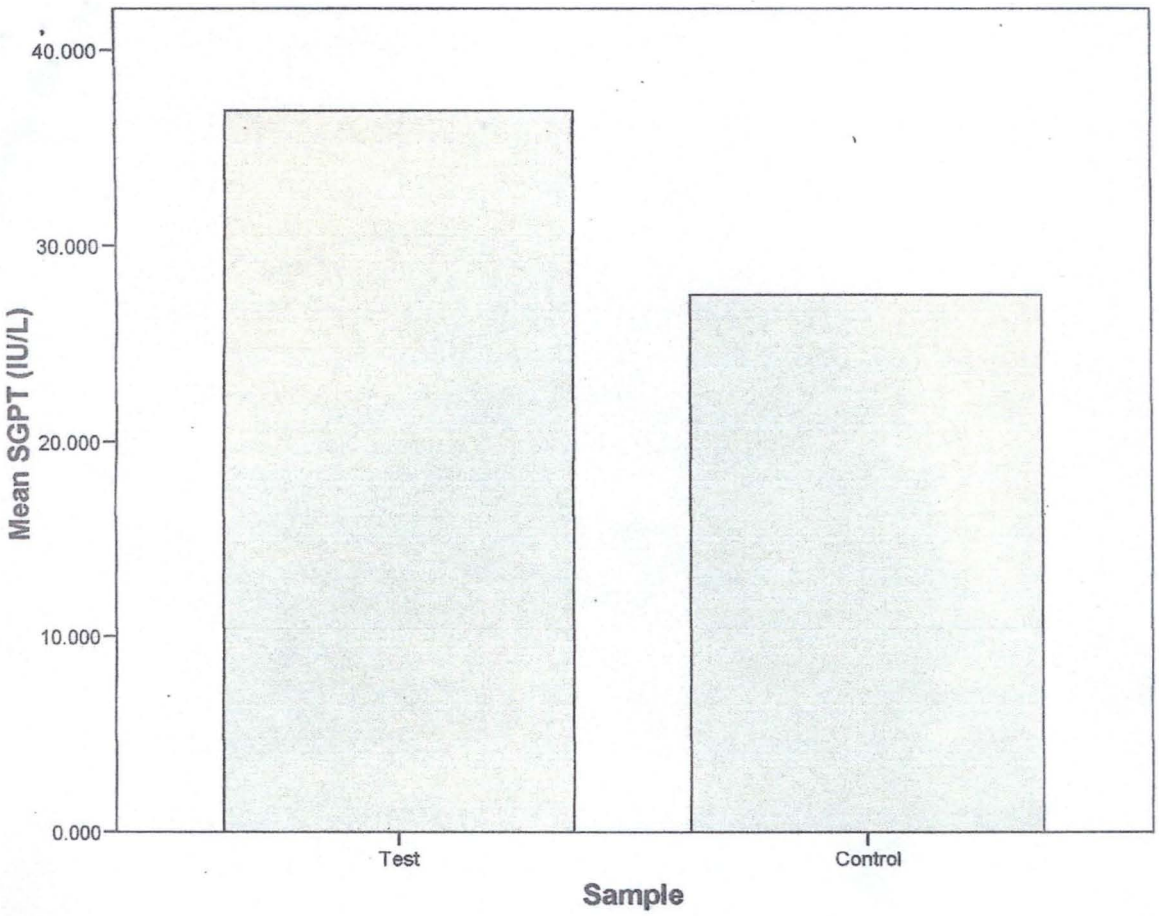
Comparison of mean level of male and female LDH activity control

Appendix IIID



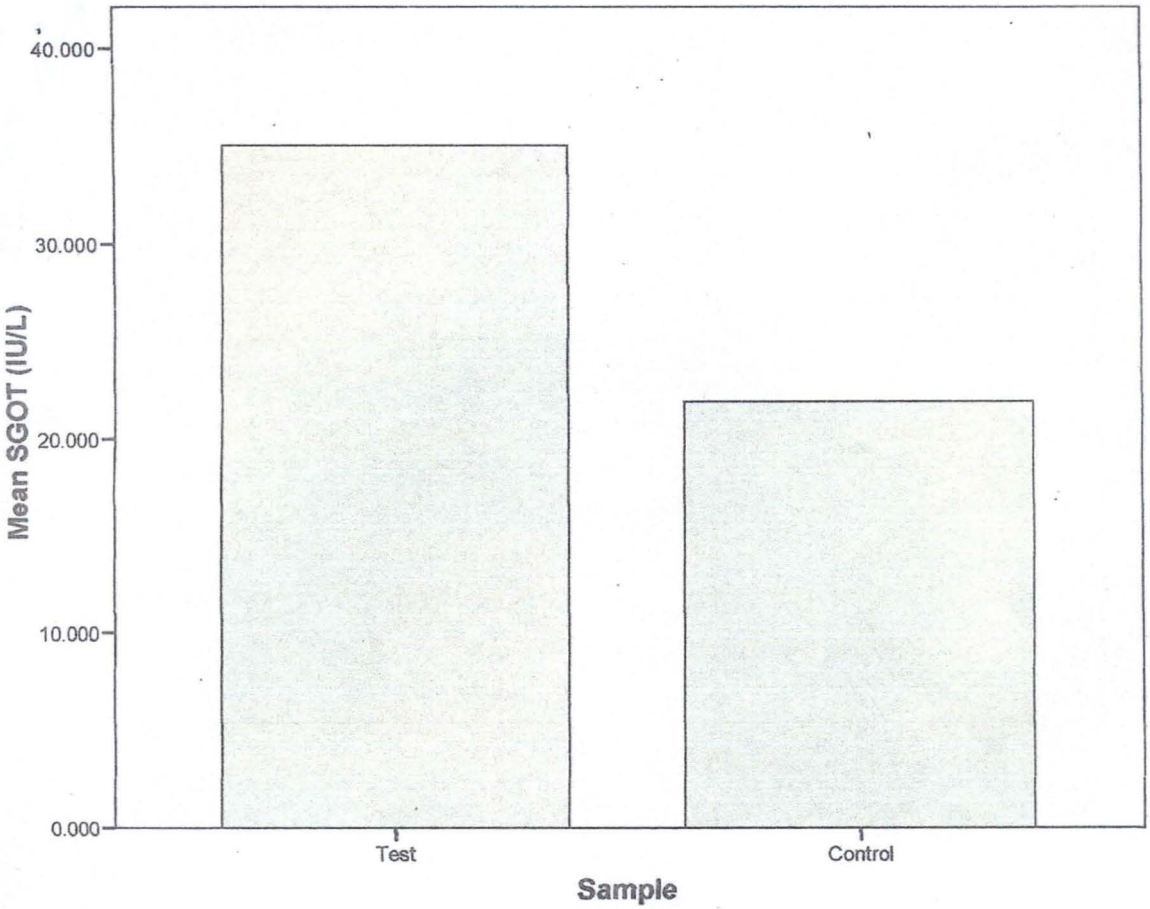
Comparison of mean level of male and female Alp activity with control

Appendix IVA



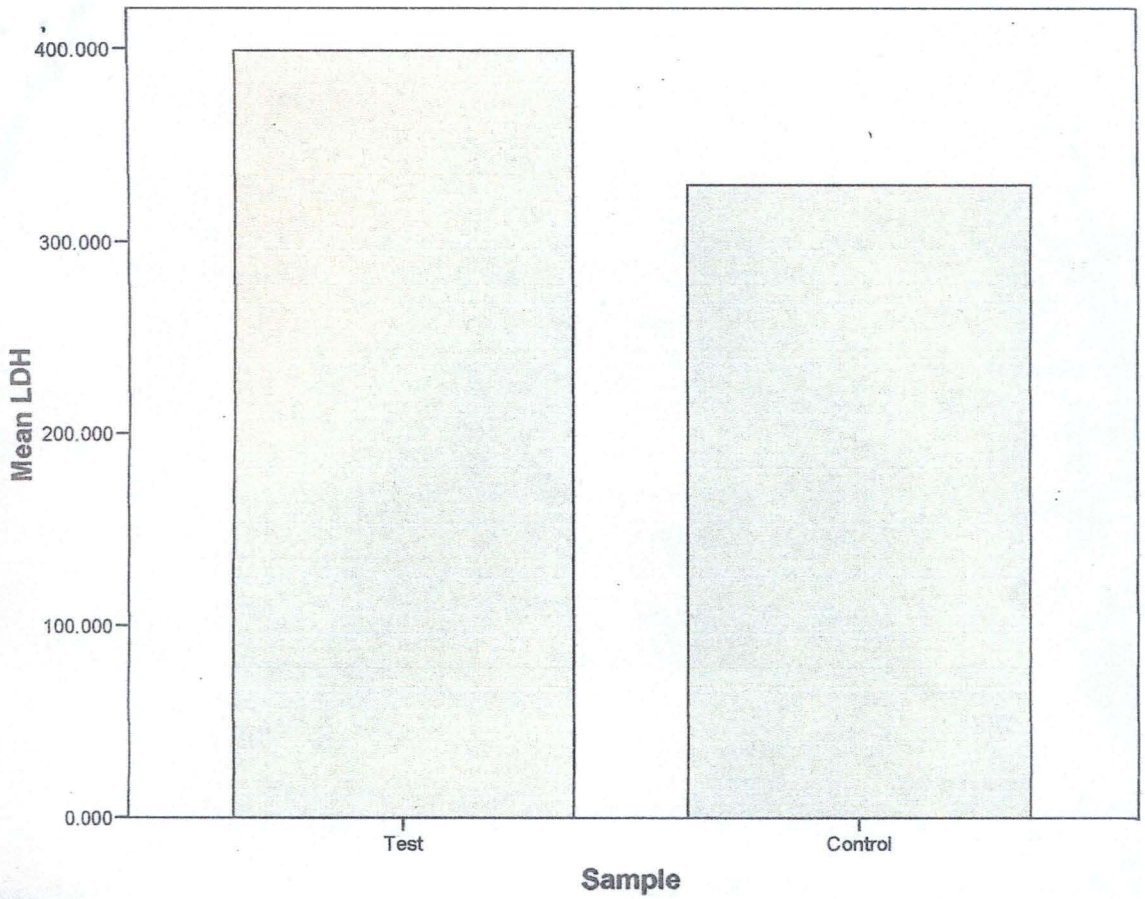
Comparison of mean sgpt activity of test and control

Appendix IVB



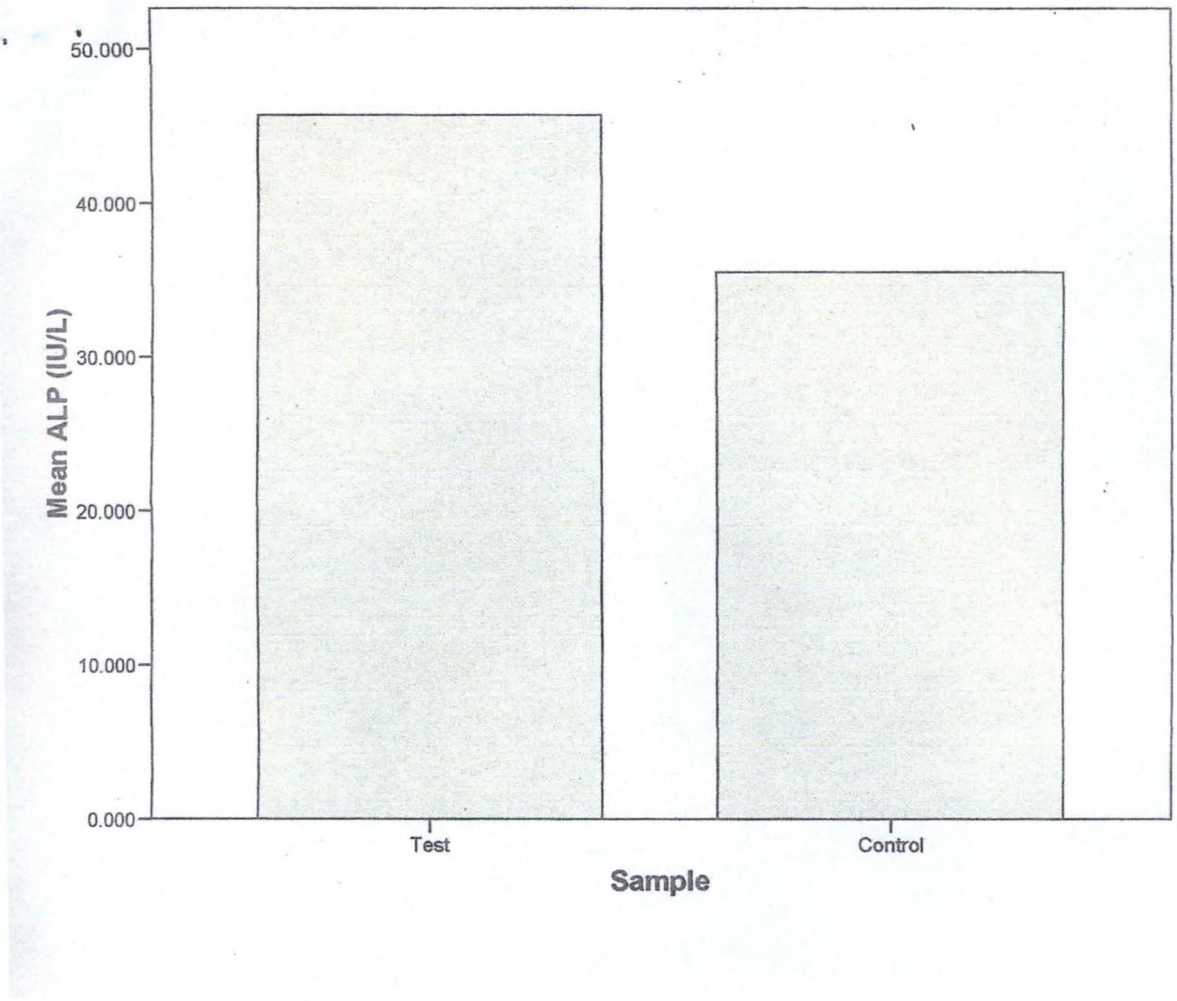
Comparison of mean sgot activity of test and control

Appendix IVC



Comparison of mean LDH activity of test and control

Appendix IVD



Comparison of mean Alp activity of test and control

Appendix VA

	Sample	Sex	PVC	Hb	G6PD	Sample1	Sex1
1	Test	Female	33.000	11.000	.020	Test	Female
2	Test	Male	31.000	10.300	.007	Test	Male
3	Test	Male	32.000	10.700	.011	Test	Male
4	Test	Male	41.000	13.700	.010	Test	Female
5	Test	Male	33.000	11.000	.009	Test	Female
6	Test	Male	35.000	11.700	.008	Test	Female
7	Test	Female	37.000	12.300	.012	Test	Female
8	Test	Female	21.000	7.000	.022	Test	Female
9	Test	Female	43.000	14.300	.026	Test	Female
10	Test	Female	25.000	8.300	.021	Test	Male
11	Test	Male	34.000	4.300	.025	Test	Male
12	Test	Female	35.000	11.700	.022	Test	Female
13	Test	Female	26.000	8.700	.014	Test	Female
14	Test	Female	30.000	10.000	.018	Test	Male
15	Test	Female	28.000	9.300	.016	Test	Male
16	Test	Female	31.000	10.300	.021	Test	Male
17	Test	Male	29.000	9.700	.012	Test	Female
18	Test	Male	27.000	9.000	.009	Test	Female
19	Test	Female	32.000	10.700	.013	Test	Male
20	Test	Male	30.000	10.000	.008	Test	Male
21	Test	Male	49.000	14.000	.009	Test	Male
22	Test	Male	32.000	10.700	.019	Test	Male
23	Test	Female	28.000	7.300	.009	Test	Female
24	Test	Female	25.000	8.300	.022	Test	Female
25	Test	Female	27.000	9.000	.017	Test	Female
26	Test	Male	32.000	10.700	.017	Test	Male
27	Test	Female	33.000	11.000	.009	Test	Male
28	Test	Female	24.000	8.000	.016	Test	Male
29	Test	Male	33.000	11.000	.019	Test	Female
30	Test	Male	41.000	13.700	.010	Test	Male
31	Test	Male	29.000	9.700	.012	Test	Female
32	Test	Male	30.000	10.000	.014	Test	Male
33	Test	Female	22.000	7.300	.012	Test	Female
34	Test	Female	23.000	7.700	.023	Test	Female
35	Test	Male	32.000	12.700	.014	Test	Male
36	Test	Male	38.000	12.700	.009	Test	Male
37	Test	Male	29.000	9.700	.005	Test	Female
38	Test	Female	27.000	9.000	.021	Test	Male

Appendix VB

	SGPT	Sample2	Sex2	SGOT	Sample3	Sex3	LDH
1	7.072	Test	Female	56.576	Test	Male	267.100
2	31.824	Test	Male	49.504	Test	Male	588.560
3	10.608	Test	Male	35.360	Test	Female	526.120
4	10.608	Test	Female	81.328	Test	Female	412.850
5	26.520	Test	Female	58.344	Test	Female	679.980
6	21.216	Test	Female	44.200	Test	Male	257.040
7	44.200	Test	Female	42.432	Test	Female	744.740
8	10.608	Test	Female	5.304	Test	Male	266.660
9	97.792	Test	Female	54.808	Test	Female	712.360
10	60.112	Test	Male	30.056	Test	Female	599.030
11	24.725	Test	Male	37.128	Test	Male	331.900
12	8.840	Test	Female	12.376	Test	Male	315.710
13	21.216	Test	Female	5.300	Test	Female	542.370
14	42.432	Test	Male	21.216	Test	Male	137.620
15	37.128	Test	Male	30.056	Test	Female	485.700
16	14.144	Test	Male	97.244	Test	Female	639.510
17	21.430	Test	Female	74.256	Test	Male	404.750
18	49.289	Test	Female	56.576	Test	Female	526.180
19	4.286	Test	Male	53.040	Test	Male	299.500
20	6.429	Test	Male	56.576	Test	Female	429.040
21	15.001	Test	Male	86.096	Test	Male	380.470
22	40.717	Test	Male	21.216	Test	Male	178.090
23	10.715	Test	Female	61.880	Test	Male	404.750
24	32.145	Test	Female	68.952	Test	Male	445.230
25	14.144	Test	Female	53.040	Test	Female	477.610
26	32.145	Test	Male	84.864	Test	Male	242.850
27	31.824	Test	Male	99.008	Test	Female	485.700
28	27.859	Test	Male	31.824	Test	Male	153.810
29	31.824	Test	Female	35.360	Test	Female	671.890
30	23.573	Test	Male	68.952	Test	Female	445.230
31	25.716	Test	Female	42.432	Test	Female	453.320
32	27.859	Test	Male	42.432	Test	Male	267.140
33	19.287	Test	Female	33.592	Test	Male	372.370
34	60.112	Test	Female	1.768	Test	Female	267.130
35	86.632	Test	Male	28.288	Test	Female	299.510
36	90.168	Test	Male	17.680	Test	Male	259.040
37	28.288	Test	Female	3.536	Test	Female	469.510
38	49.504	Test	Male	1.768	Test	Female	323.800

Appendix VC

	Sample4	Sex4	ALP
1	Test	Female	18.300
2	Test	Female	30.400
3	Test	Female	39.600
4	Test	Female	45.600
5	Test	Female	46.700
6	Test	Male	36.500
7	Test	Male	38.000
8	Test	Male	31.400
9	Test	Female	13.300
10	Test	Female	38.500
11	Test	Male	68.000
12	Test	Female	42.600
13	Test	Female	55.800
14	Test	Male	53.800
15	Test	Male	58.800
16	Test	Male	50.700
17	Test	Female	28.400
18	Test	Female	56.300
19	Test	Female	28.400
20	Test	Female	30.400
21	Test	Male	42.600
22	Test	Female	36.500
23	Test	Male	65.900
24	Test	Male	52.700
25	Test	Male	25.300
26	Test	Female	48.600
27	Test	Female	55.700
28	Test	Female	41.500
29	Test	Male	71.000
30	Test	Male	51.700
31	Test	Male	44.600
32	Test	Male	42.600
33	Test	Male	34.400
34	Test	Male	57.800
35	Test	Female	41.500
36	Test	Female	49.700
37	Test	Female	24.300
38	Test	Female	59.800

Appendix VD

	Sample	Sex	PVC	Hb	G6PD	Sample1	Sex1
39	Test	Female	32.000	10.700	.010	Test	Female
40	Test	Male	36.000	12.000	.011	Test	Male
41	Test	Male	24.000	8.000	.018	Test	Male
42	Test	Female	39.000	13.000	.016	Test	Male
43	Test	Female	28.000	9.300	.008	Test	Female
44	Test	Female	38.000	11.000	.013	Test	Female
45	Test	Female	28.000	9.300	.012	Test	Female
46	Test	Female	25.000	8.300	.014	Test	Female
47	Test	Female	31.000	10.300	.015	Test	Male
48	Test	Male	33.000	11.000	.013	Test	Male
49	Control	Male	44.000	14.700	.008	Test	Male
50	Control	Male	42.000	14.000	.010	Test	Male
51	Control	Male	40.000	13.300	.025	Test	Male
52	Control	Female	37.000	12.300	.016	Test	Female
53	Control	Male	23.000	7.700	.007	Test	Female
54	Control	Male	42.000	14.000	.014	Test	Male
55	Control	Male	31.000	10.300	.018	Test	Male
56	Control	Male	28.000	9.300	.008	Test	Female
57	Control	Male	39.000	13.000	.011	Test	Female
58	Control	Female	32.000	10.700	.012	Test	Female
59	Control	Male	34.000	11.300	.013	Test	Female
60	Control	Female	31.000	10.300	.014	Test	Female
61						Test	Male
62						Test	Male
63						Test	Male
64	.	.	.	.	.	Test	Male
65						Test	Male
66						Test	Female
67						Test	Female
68						Test	Female
69	.	.	.	.	.	Test	Female
70						Test	Male
71	.	.	.	.	.	Test	Female
72						Test	Male
73	.	.	.	.	.	Test	Female
74	.	.	.	.	.	Test	Female
75	.	.	.	.	.	Test	Female
76						Test	Male

Appendix VE

	SGPT	Sample2	Sex2	SGOT	Sample3	Sex3	LDH
39	79.560	Test	Female	5.304	Test	Male	234.760
40	47.736	Test	Male	15.912	Test	Male	226.660
41	76.024	Test	Male	22.984	Test	Female	267.130
42	56.576	Test	Male	7.072	Test	Male	380.470
43	54.808	Test	Female	5.304	Test	Male	267.130
44	42.432	Test	Female	5.304	Test	Female	.
45	44.200	Test	Female	17.678	Test	Female	445.220
46	49.504	Test	Female	54.808	Test	Male	331.900
47	37.128	Test	Male	31.824	Test	Female	.
48	61.880	Test	Male	21.216	Test	Female	429.040
49	51.272	Test	Male	31.824	Control	.	202.380
50	47.736	Test	Female	10.608	Control	.	339.990
51	40.664	Test	Male	19.303	Control	.	493.800
52	40.664	Test	Male	32.145	Control	.	348.080
53	84.864	Test	Female	15.001	Control	.	542.370
54	44.200	Test	Female	17.144	Control	.	477.610
55	26.520	Test	Female	8.572	Control	.	323.800
56	26.520	Test	Male	17.144	Control	.	388.700
57	60.112	Test	Female	27.859	Control	.	234.760
58	26.520	Test	Male	42.860	Control	.	194.280
59	38.896	Test	Female	29.020	Control	.	250.940
60	44.200	Test	Female	42.860	Control	.	275.200
61	49.504	Test	Male	38.570	Control	.	275.230
62	23.573	Test	Male	66.433	Control	.	250.950
63	40.717	Test	Male	72.862	Control	.	339.990
64	56.576	Test	Female	15.001	.	.	.
65	40.717	Test	Male	32.145	.	.	.
66	76.024	Test	Male	30.002	.	.	.
67	23.573	Test	Female	21.430	.	.	.
68	14.144	Test	Female	19.287	.	.	.
69	12.376	Test	Male	19.287	.	.	.
70	22.984	Test	Female	5.300	.	.	.
71	34.128	Test	Female	42.432	.	.	.
72	37.128	Test	Male	21.430	.	.	.
73	30.056	Test	Male	21.430	.	.	.
74	17.680	Test	Male	17.144	.	.	.
75	38.896	Test	Male	49.504	.	.	.
76	51.272	Test	Female	33.582	.	.	.

Appendix VF

	Sample4	Sex4	ALP
39	Test	Male	43.600
40	Test	Male	49.700
41	Test	Female	49.700
42	Test	Female	47.600
43	Test	Male	42.600
44	Test	Male	51.700
45	Test	Female	59.800
46	Test	Female	38.500
47	Test	Male	57.800
48	Test	Male	58.800
49	Test	Female	40.500
50	Test	Male	69.900
51	Test	Male	54.700
52	Test	Male	52.700
53	Test	Female	59.800
54	Test	Female	46.600
55	Test	Male	34.400
56	Control	.	43.600
57	Control	.	31.400
58	Control	.	33.400
59	Control	.	54.700
60	Control	.	27.300
61	Control	.	20.200
62	Control	.	36.500
63	Control	.	29.400
64	Control	.	39.500
65	Control	.	24.300
66	Control	.	29.400
67	Control	.	32.400
68	Control	.	32.400
69	Control	.	26.300
70	Control	.	34.400
71	Control	.	67.900
72	Control	.	43.600
73	Control	.	44.600
74	Control	.	48.600
75	Control	.	19.300
76	Control	.	27.300

Appendix VG

	Sample	Sex	PVC	Hb	G6PD	Sample1	Sex1
77	.	.	.	.	.	Test	Male
78	.	.	.	.	.	Test	Female
79	.	.	.	.	.	Test	Female
80	.	.	.	.	.	Test	Female
81	.	.	.	.	.	Test	Female
82	.	.	.	.	.	Test	Female
83	.	.	.	.	.	Test	Male
84	.	.	.	.	.	Test	Male
85	.	.	.	.	.	Test	Female
86	.	.	.	.	.	Test	Male
87	.	.	.	.	.	Test	Female
88	.	.	.	.	.	Test	Male
89	.	.	.	.	.	Test	Female
90	.	.	.	.	.	Test	Male
91	.	.	.	.	.	Test	Female
92	.	.	.	.	.	Test	Female
93	.	.	.	.	.	Test	Female
94	.	.	.	.	.	Test	Male
95	.	.	.	.	.	Test	Male
96	.	.	.	.	.	Test	Male
97	.	.	.	.	.	Test	Female
98	.	.	.	.	.	Control	.
99	.	.	.	.	.	Control	.
100	.	.	.	.	.	Control	.
101	.	.	.	.	.	Control	.
102	.	.	.	.	.	Control	.
103	.	.	.	.	.	Control	.
104	.	.	.	.	.	Control	.
105	.	.	.	.	.	Control	.
106	.	.	.	.	.	Control	.
107	.	.	.	.	.	Control	.
108	.	.	.	.	.	Control	.
109	.	.	.	.	.	Control	.
110	.	.	.	.	.	Control	.
111	.	.	.	.	.	Control	.
112	.	.	.	.	.	Control	.
113	.	.	.	.	.	Control	.
114	.	.	.	.	.	Control	.

Appendix VH

	SGPT	Sample2	Sex2	SGOT	Sample3	Sex3	LDH
77	45.968	Test	Female	22.288	.	.	.
78	28.288	Test	Female	31.824	.	.	.
79	40.664	Test	Male	53.040	.	.	.
80	23.573	Test	Female	42.860	.	.	.
81	42.432	Test	Male	67.184	.	.	.
82	17.680	Test	Female	22.984	.	.	.
83	7.072	Test	Female	31.824	.	.	.
84	53.040	Test	Male	15.001	.	.	.
85	26.520	Test	Male	22.984	.	.	.
86	63.648	Test	Female	25.716	.	.	.
87	49.504	Test	Female	53.040	.	.	.
88	35.360	Test	Female	56.576	.	.	.
89	86.632	Test	Male	23.573	.	.	.
90	38.896	Test	Male	51.272	.	.	.
91	37.128	Test	Female	49.504	.	.	.
92	44.200	Test	Female	17.144	.	.	.
93	23.573	Test	Female	15.001	.	.	.
94	12.376	Test	Female	37.128	.	.	.
95	10.608	Test	Male	23.573	.	.	.
96	30.056	Test	Male	51.272	.	.	.
97	15.912	Test	Male	49.504	.	.	.
98	1.768	Test	Female	10.608	.	.	.
99	14.144	Test	Male	42.432	.	.	.
100	10.668	Test	Male	42.860	.	.	.
101	12.376	Test	Female	8.840	.	.	.
102	21.216	Control	.	25.716	.	.	.
103	14.144	Control	.	34.288	.	.	.
104	21.216	Control	.	25.716	.	.	.
105	24.752	Control	.	12.858	.	.	.
106	32.360	Control	.	17.144	.	.	.
107	19.448	Control	.	10.715	.	.	.
108	49.504	Control	.	19.287	.	.	.
109	47.736	Control	.	15.001	.	.	.
110	38.896	Control	.	19.287	.	.	.
111	28.288	Control	.	17.144	.	.	.
112	53.640	Control	.	8.572	.	.	.
113	40.664	Control	.	17.144	.	.	.
114	60.112	Control	.	21.430	.	.	.

Appendix VI

	Sample4	Sex4	ALP
77	.	.	.
78	.	.	.
79	.	.	.
80	.	.	.
81	.	.	.
82	.	.	.
83	.	.	.
84	.	.	.
85	.	.	.
86	.	.	.
87	.	.	.
88	.	.	.
89	.	.	.
90	.	.	.
91	.	.	.
92	.	.	.
93	.	.	.
94	.	.	.
95	.	.	.
96	.	.	.
97	.	.	.
98	.	.	.
99	.	.	.
100	.	.	.
101	.	.	.
102	.	.	.
103	.	.	.
104	.	.	.
105	.	.	.
106	.	.	.
107	.	.	.
108	.	.	.
109	.	.	.
110	.	.	.
111	.	.	.
112	.	.	.
113	.	.	.
114	.	.	.

Appendix VJ

	Sample	Sex	PVC	Hb	G6PD	Sample1	Sex1
115	.	.	.	.	.	Control	.
116	.	.	.	.	.	Control	.
117	.	.	.	.	.	Control	.
118	.	.	.	.	.	Control	.
119	.	.	.	.	.	Control	.
120	.	.	.	.	.	Control	.
121	.	.	.	.	.	Control	.
122	.	.	.	.	.	Control	.
123	.	.	.	.	.	Control	.
124	.	.	.	.	.	Control	.
125	.	.	.	.	.	Control	.
126	.	.	.	.	.	Control	.
127	.	.	.	.	.	Control	.
128	.	.	.	.	.	Control	.
129	.	.	.	.	.	Control	.
130	.	.	.	.	.	Control	.
131	.	.	.	.	.	.	.
132	.	.	.	.	.	.	.
133	.	.	.	.	.	.	.
134	.	.	.	.	.	.	.
135	.	.	.	.	.	.	.
136	.	.	.	.	.	.	.
137	.	.	.	.	.	.	.
138	.	.	.	.	.	.	.
139	.	.	.	.	.	.	.
140	.	.	.	.	.	.	.
141	.	.	.	.	.	.	.

Appendix VK

	SGPT	Sample2	Sex2	SGOT	Sample3	Sex3	LDH
115	14.144	Control	.	15.001	.	.	.
116	19.448	Control	.	23.573	.	.	.
117	21.216	Control	.	21.430	.	.	.
118	15.912	Control	.	25.716	.	.	.
119	33.592	Control	.	10.715	.	.	.
120	38.896	Control	.	23.573	.	.	.
121	14.144	Control	.	17.144	.	.	.
122	19.448	Control	.	25.716	.	.	.
123	10.608	Control	.	3.536	.	.	.
124	56.576	Control	.	28.288	.	.	.
125	33.592	Control	.	44.200	.	.	.
126	37.128	Control	.	42.430	.	.	.
127	12.376	Control	.	31.824	.	.	.
128	31.824	Control	.	30.056	.	.	.
129	28.288	Control	.	24.752	.	.	.
130	30.056	Control	.	15.000	.	.	.
131	.	Control	.	35.360	.	.	.
132	.	Control	.	28.230	.	.	.
133	.	Control	.	23.573	.	.	.
134	.	Control	.	23.570	.	.	.
135	.	Control	.	22.984	.	.	.
136	.	Control	.	23.573	.	.	.
137	.	Control	.	24.752	.	.	.
138	.	Control	.	17.144	.	.	.
139	.	Control	.	17.144	.	.	.
140	.	Control	.	21.430	.	.	.
141	.	Control	.	10.608	.	.	.

Appendix VL

	SGPT	Sample2	Sex2	SGOT	Sample3	Sex3	LDH
115	14.144	Control	.	15.001	.	.	.
116	19.448	Control	.	23.573	.	.	.
117	21.216	Control	.	21.430	.	.	.
118	15.912	Control	.	25.716	.	.	.
119	33.592	Control	.	10.715	.	.	.
120	38.896	Control	.	23.573	.	.	.
121	14.144	Control	.	17.144	.	.	.
122	19.448	Control	.	25.716	.	.	.
123	10.608	Control	.	3.536	.	.	.
124	56.576	Control	.	28.288	.	.	.
125	33.592	Control	.	44.200	.	.	.
126	37.128	Control	.	42.430	.	.	.
127	12.376	Control	.	31.824	.	.	.
128	31.824	Control	.	30.056	.	.	.
129	28.288	Control	.	24.752	.	.	.
130	30.056	Control	.	15.000	.	.	.
131	.	Control	.	35.360	.	.	.
132	.	Control	.	28.230	.	.	.
133	.	Control	.	23.573	.	.	.
134	.	Control	.	23.570	.	.	.
135	.	Control	.	22.984	.	.	.
136	.	Control	.	23.573	.	.	.
137	.	Control	.	24.752	.	.	.
138	.	Control	.	17.144	.	.	.
139	.	Control	.	17.144	.	.	.
140	.	Control	.	21.430	.	.	.
141	.	Control	.	10.608	.	.	.