"VARIATION IN COMMON LIPID PARAMETERS IN MALARIA INFECTED PATIENTS - A ONE YEAR CROSS SECTIONAL STUDY"

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ENDORSEMENT

This is to certify that the dissertation entitled **"VARIATION IN COMMON LIPID PARAMETERS IN MALARIA INFECTED PATIENTS - A ONE YEAR CROSS SECTIONAL STUDY"** is a bonafide research work done by **REG NO. BG0112008.**

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LIST OF ABBREVIATIONS USED

µl	-	Microliter
AIDS	-	Acquired immunodeficiency syndrome
ALT	-	Alanine amino transferase
ANOVA	-	Analysis of variance
ARDS	-	Acute respiratory distress syndrome
AST	-	Aspartate amino transferase
B.C.	-	Before Christ
BP	-	Blood pressure
CNS	-	Central nervous system
CPK	-	Creatine phosphokinase
CSF	-	Cerebrospinal fluid
DIC	-	Disseminated intravascular coagulation
DNA	-	Deoxyribonucleic acid
EBV	-	Epstein-Barr virus
ELISA	-	Enzyme linked immunosorbant assay
FFA	-	Free fatty acids
G6PD	-	Glucose-6-phosphate dehydrogenase
GI	-	Gastro-intestinal
GIT	-	Gastro-intestinal tract
HAART	-	Highly active antiretroviral therapy
Hb	-	Hemoglobin
HCT	-	Hematocrit
HDL	-	High-density lipoprotein
HLA	-	Human leukocyte antigen

Hr	-	Hour
ICAM	-	Inter cellular adhesion molecule
IgG	-	Immunoglobulin G
IgM	-	Immunoglobulin M
IL	-	Interleukin
IM	-	Intramuscular
IV	-	Intravenous
SC	-	Subcutaneous
IU/L	-	International units per litre
Kg	-	Kilogram
mg	-	Milligram
mm Hg	-	Millimeters of mercury
mmol	-	Millimole
MP	-	Malarial parasite
n	-	Total number
NADP	-	Nicotinamide adenine dinucleotide phosphate
NCEP	-	National Cholesterol Education Program
NMCP	-	National Malaria Control Program
NVBDCP	-	National Vector Borne Disease Control Program
^{o}C	-	Degree centigrade
p	-	Probability value
P. falciparum	-	Plasmodium falciparum
PCV	-	Packed cell volume
$PFEMP_1$	-	Plasmodium falciparum erythrocyte memberane protein 1
PI	-	Parasite index

PVM	-	parasitophorous vacuolar membrane
QBC	-	Quantitative buffy coat
RBC	-	Red blood cell
RBS	-	Random blood sugar
RDT	-	Rapid diagnostic test
RE	-	Reticuloendothelial
RNA	-	Ribonucleic acid
ROS	-	Reactive oxygen species
SD	-	Standard deviation
SEA	-	South East Asia
SGOT	-	serum glutamic oxaloacetic transaminase
SGPT	-	serum glutamic-pyruvic transaminase
TC	-	Total cholesterol
TNF	-	Tumor necrosis factor
UV	-	Ultravoilet
VLDL	-	Very low-density lipoprotein
WHO	-	World Health Organization
µmol	-	Micromole

ABSTRACT

Background and objectives

Malaria is an important infectious disease and serum lipid profile changes have been observed during the course of infection. The present study was aimed to assess the lipid profile in patients with malaria infection.

Methodology

This one year cross sectional study was done from January 2013 to December 2013 in the Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum. A total of 50 adult patients with clinical features of malaria infection were investigated for serum lipid profile changes.

Results

Majority of the patients were males (82%) with male to the female ratio 4.5:1. The mean age was 33.96 ± 12.72 years. At presentation all the patients had fever with chills and rigors (100%) while generalized body pain was noted in 80% and the commonest clinical sign was pallor (40%). Systemic examination revealed, splenomegaly in 40%. Majority (86%) of the patients were positive for Plasmodium vivax. Thrombocytopenia was noted in 64% of the patients. Serum lipid profile estimation in these patients revealed that 60% of the patients had low total cholesterol levels (100 to 150 mg/dL), 56% of them had low LDL levels with value being less than 50 mg/dL and , 58% of patients had low HDL levels (<20 mg/dL) whereas 92% of the patients with hypertriglyceridemia (>150

mg/dL). Incidentally we found that patients with low LDL cholesterol level had low platelet count (51.25±29.67; p=0.035).

Conclusion

Patients with malaria infections may have lipid parameters alteration in terms of hypocholesterolemia, low HDL, low LDL levels and hypertriglyceridemia.

Keywords

Hypertriglyceridemia; Hypocholesterolemia; Hypolipidemia; Malaria infection; Serum lipid profile;

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Chapter 1

Introduction

INTRODUCTION

Malaria, sometimes called the "King of Diseases", is an important parasitic infection with considerable morbidity and mortality.[1] This disease was almost eradicated in 1960's and has re-emerged as a major public health problem in the last few decades.[2]

Malaria is an important infectious disease in tropical and subtropical regions and continues to be a major global health problem with more than 2.4 billion people (over 40% of the world's population) being exposed to varying degrees of malaria risk in some 100 countries in the tropics from South America to the Indian peninsula. The tropics provide ideal breeding and living conditions for the anopheles mosquito and hence this distribution.[3]

Every year 300 million to 500 million people suffer from this disease (90% of them in sub-Saharan Africa, two thirds of the remaining cases occur in six countries- India, Brazil, Sri Lanka, Vietnam, Colombia and Solomon Islands). World Health Organization (WHO) forecasts a 16% growth in malaria cases annually. About 1.5 million to 3 million people die of malaria every year (85% of these occur in Africa), accounting for about 4-5% of all fatalities in the world. One child dies of malaria somewhere in Africa every 20 second and there is one malarial death every 12 second somewhere in the world. Each year malaria kills same number of people as AIDS does in 15 years. In 15 years, if 5 million have died of AIDS, 50 million have had died of malaria.[3]

Malaria ranks third among the major infectious diseases in causing deaths- after pneumococcal acute respiratory infections and tuberculosis. It is

expected that by the turn of the century malaria would be the number one infectious killer disease in the world. It accounts for 2.6 percent of the total disease burden of the world. It is responsible for the loss of more than 35 million disability-adjusted life-years each year. Every year around 30000 visitors to endemic areas develop malaria and 1% of them die.[4]

Malaria is caused by protozoan parasites of the genus *Plasmodium*. The most serious and sometimes fatal type of malaria is caused by *Plasmodium falciparum (P. falciparum)*. The other human malaria species, *Plasmodium vivax (P. vivax), Plasmodium ovale, Plasmodium malariae*, and sometimes *Plasmodium knowlesi* can cause acute, severe illness but mortality rates are low.[1] Since 1994 focal outbreaks of malaria have been reported from various parts of India with increased proportion of P. *falciparum*.

The number of malaria cases worldwide seems to be increasing due to increase in transmission risk, prevalence of drug resistant strains of the parasite and international travel and migration.[5]

Patients with malaria often exhibit various laboratory abnormalities due to an acute phase response, but little is known about lipid profile changes in malaria infected patients.

In 1978, Lambrecht et al.[6] reported transient lipid profile changes in six returning travelers with malaria infection caused by *Plasmodium vivax* and suggested for the first time that changes in high-density lipoprotein (HDL) in human serum are related to the lipid metabolism of the parasite. It was

hypothesized that the malaria parasite uses cholesterol and phospholipids from its host resulting in a decrease of serum HDL.

Prior to this report, Angus et al.[7] utilized lipoprotein electrophoresis in rhesus monkeys infected with *Plasmodium knowlesi* to study serum lipids in malaria. Their results were not conclusive because lipoprotein bands could barely be detected in the serum of controls. Subsequently, several clinical studies showed lipid profile changes in the setting of both uncomplicated and complicated malaria.[8-13]

During intraerythrocytic stage in humans, malaria parasite actively internalizes phospholipids from erythrocyte membrane and the extracellular medium. The import of exogenous lipids is not due to endocytosis; is energy dependent trans bilayer movement of phospholipids induced by the parasite in the erythrocyte surface.[14]

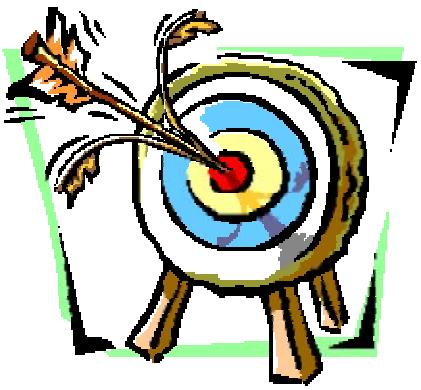
Although the magnitude of changes seems to be related to the severity of malaria in several studies, others found no correlation between the severity of malaria attacks and the extent of lipid profile changes. These transient lipid profile changes in malaria infection have been suggested by some researchers as a potential adjuvant diagnostic tool for malaria.[15]

The extent of serum lipid profile changes during malaria infection and their underlying biological mechanisms remain unclear. Mechanism may be partly host related (i e, related to an acute phase reaction), parasite-related, or a combination of these two.[15]

Therefore, it is hypothesized that the lipid profile in malaria infection exhibits characteristic changes. However, if a link between human host serum lipid alterations and the pathogenesis of malaria can be demonstrated, further studies to elucidate the precise pathways can be conducted. Moreover, novel treatment approaches could be explored with lipid metabolism-regulating drugs. In addition, it is understood that these changes are specific for the malaria pathogen-host interplay. Hence the present study was planned to assess the lipid profile in malaria infected patients.

Chapter 2

Objectives



OBJECTIVES

The objective of present study was to assess the lipid profile in malaria infected patients.

Chapter 3

Review of Literature

REVIEW OF LITERATURE

Historical perspectives

Malaria is the most important protozoan infection of the red blood cells in humans and is transmitted by the bite of blood feeding female anopheles mosquito. The word “malaria” or “ague” has been known from antiquity. It is derived from the Roman word means literally “Bad Air”. It was supposed to be due to offensive vapours emanating from tiberian marshes.[16]

Hippocrates (400 B.C.) was credited for the first clear description of pattern of fever and in his aphorisms he described about the regular paroxysms of intermittent fever.

A French army surgeon working in Algeria was the first to come out with an information that parasites caused malaria.[17] On 20th October 1880 Charles Louis Alphonse Laveran who was examining the fresh blood of a patient with ague observed moving bodies (Probably watching gametocytes exflagellation) called parasites of red blood cell[17]. The transmission of infection in blood was proved by Gerbardt. The vector transmission of the plasmodium falciparum was first described by a young Scottish military physician Sir Ronald Ross on Aug. 20th 1897 while he was working in Secundarabad India and later in Calcutta.[18]

The complete life cycle was described by Ross. For their contribution in the investigation of malaria both Ross and Laveran received Nobel prizes. In Rome Seirraleone Bignami et al. confirmed the sporogony in anopheles

mosquito and they succeeded in infecting a healthy volunteer with falciparum from mosquito bite.[19]

Epidemiology

Prevalence

Worldwide

Malaria is one of the most important public health problem in term of morbidity and mortality, causing more than 200 million cases every year.[20] According to the WHO Malaria Report 2011, a total of 106 countries in the world are at risk of transmission of malaria infection.[21]

A total of 216 million estimated malaria cases occurred in 2010, 81% of which were reported in the African Region, followed by South East Asia (13%) and Eastern Mediterranean Region (5%).[20]

Figure 1. Malaria, countries or areas at risk of transmission (2010)[21]

Although the proportion of people exposed to malaria parasites has decreased during the last century, the absolute number of people at risk for malaria infection increased from 0.8 billion in 1900 to 3.3 billion in 2010, as a consequence of the absolute increase in the population living in malaria-endemic areas.[21,22]

Malaria is most prevalent in rural tropical areas less than 1000 m (3282 ft) elevation but is not limited to these climates. *P falciparum* is found mostly in the tropics and accounts for about 50% of cases and 95% of malarial deaths worldwide. *P vivax* is distributed more widely than *P falciparum,* but it causes less morbidity and mortality however, both *P vivax* and *P ovale* can establish a hypnozoite phase in the liver resulting in latent infection.[23]

India

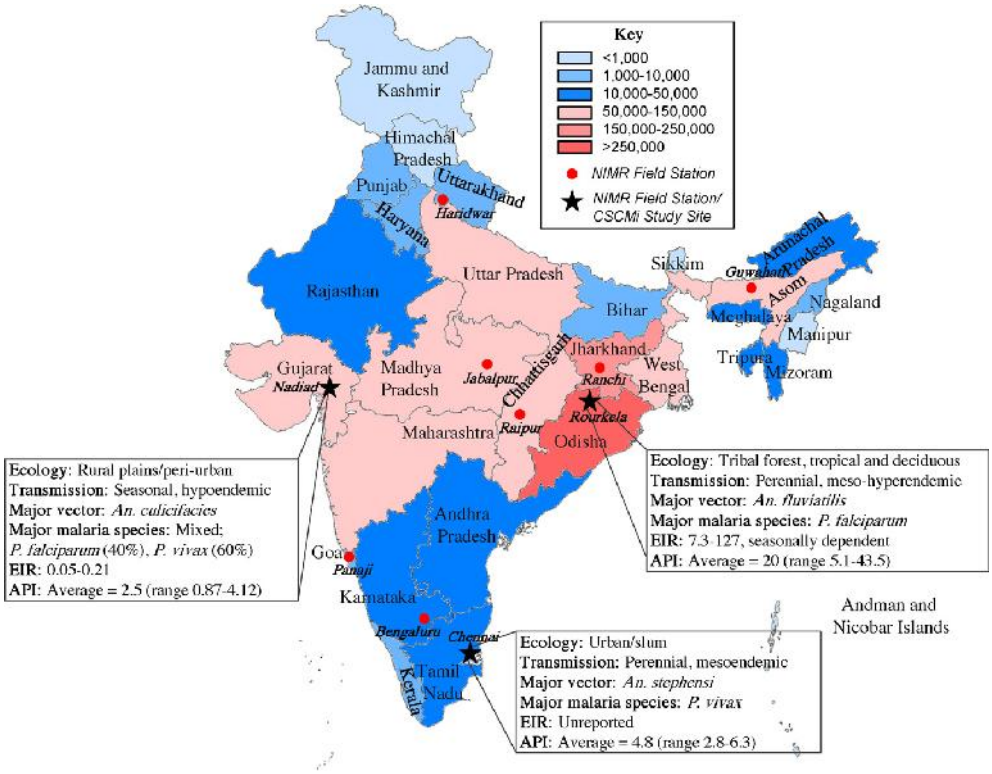


Figure 2. Malaria endemicity in India[24]

Malaria is a major public health problem in India and the one which contributes significantly to the overall malaria burden in Southeast Asia.

In India, based on provisional epidemiological data from Directorate of National Vector Borne Disease Control Program statistics for the year 2013 and 2014, there were 368 deaths with 18.6% increase in number of cases compared to 2012 statistics[25].

As the second most populous country in the world, with a population exceeding one billion people, India's public health system faces many challenges including implementation of surveillance programs to accurately estimate and control the national malaria burden. Historically, the highest incidence of malaria in India occurred in the 1950s, with an estimated 75 million cases and 0.8 million deaths per year (WHO, Country Office for India). The launch of the National Malaria Control Program (NMCP) in 1953 resulted in a significant decline in the number of reported cases to <50,000 and reported no mortality, by 1961. Despite its near elimination in the mid-1960s, malaria resurged to ≈6.45 million cases in 1976. Since then, confirmed cases have gradually decreased to 1.6 million cases and ≈1100 deaths in 2009.[24]

Currently, 80.5% of the 1.2 billion population of India lives in malaria risk areas. Of this, 4.2%, 32.5% and 43.8% live in areas of high, moderate and low risk to malaria respectively.[26] At present, official figures for malaria in India, available at NVBDCP,[27] indicate 1.5–2 million confirmed cases and about 1,000 deaths annually.[28] According to the WHO South East Asia Regional Office estimates, during 2000-2009, malaria incidence remained between the range 2.16

-2.83 millions and malaria deaths between 3188 - 6978 in SEA Region, the proportion *of P. falciparum* being 44 – 60% and more than 70% of these cases being reported from India. During 2009, total 2.7 million confirmed malaria cases (Microscopically and RDT) and 3188 malaria deaths were reported in the SEAR countries where as estimated malaria cases were around 26 -36 million and malaria deaths between 42300 – 77300. The *P. falciparum* proportion remained around 60.5% (including RDT positives). Of these, the highest number of laboratory confirmed cases were reported from India .[29]

Sex-related demographics

Males and females are affected equally. However, malaria may be devastating during pregnancy to the mother and the fetus. *P falciparum* is the primary malaria species responsible for increased morbidity and mortality in pregnancy. The prevalence of malaria is higher in primigravidas than in nonpregnant women or multigravidas.[30]

Maternal complications are thought to be mediated by pregnancy associated decreases in immune function, as well as by placental sequestration of (*P falciparum*) parasites. Anemia from malaria can be more severe in pregnant women. Fetal complications include premature birth, anemia, low birth weight and death. Malaria during the first trimester of pregnancy increases the risk for miscarriage.[31]

Age-related demographics

Young children aged 6 months to 3 years who live in endemic areas are at an increased risk of death due to malaria. Travelers without immunity are at an increased mortality risk, regardless of age.

Seasonal variation

Malaria is often seasonal coinciding with the rainy season which provides water for mosquito breeding and increased humidity favouring mosquito survival. Malaria transmission does not occur at temperatures below 16°C or above 33°C and at altitudes greater than 2000 metre as development in the mosquito (sporogony) cannot take place. The optimum conditions for transmission are high humidity and an ambient temperature between 20 to 30°C.

Transmission

Female Anopheles stephensi is the principal vector of transmission of malaria in India.[28] Anopheles culicifacies and Anopheles fluviatilis are more common in rural areas and foothills of South lndia.[32]

Malaria transmission to man depends on following factors[33]

1. Longevity of anopheles mosquito: This is needed as sporogony takes over a week. The mosquito must survive for longer than this after feeding on a gametocyte carrying human if malaria is to be transmitted.

2. Density of vector: Malaria transmission is directly proportional to the density of the vector, the square of the number of times each day that the mosquito bites man and the 10^{th} power of the probability of the mosquito surviving for one day - model described by McDonald though has certain theoretical limitation useful in control of the diseases.

e.g., Malaria transmission = K x density of vector (D) x Square of number of times each day the mosquito bites man (S^2) x 10th power of the probability of the mosquito survival for one day (p^{10})**,** where K is constant derived for that endemic area.[32]

Human host

Human reservoir of gametocytes is required to transmit the infection. In areas of high transmission infants and young children are more susceptible than the adults. Younger age groups have high parasite density than old age. The older age group have asymptomatic infection and low parasite density due to immunity. Endemicity of malaria is defined traditionally in terms of spleen or parasite rates in children aged 2 to 9 years.[34]

Hypo endemic - Spleen or parasite rate 0 - 10%.

Meso endemic - Spleen or parasite rate 10 - 50%.

Hyper endemic - Spleen or parasite rate 50 - 75%.

Holo endemic - Spleen or parasite rate> 75%

Where parasite rate is defined as the percentage of children between the age of 2-9 years showing malaria parasites in their blood films in that particular area. The spleen rate is the percentage of children between 2-9 years of age having an enlarged spleen.

Falciparum malaria infection are more severe in non immune persons, and in pregnancy particularly primigravida and may be augmented by Iron supplementation.[35] In newborn babies, malaria is infrequent because of:

1. Passive transfer of immunity from the mother.
2. Presence of Hb F in infant RBC which retards the parasite development.

Malaria may also be transmitted by blood transfusion, bone marrow transplantation or through needle sharing among intravenous drug addicts.

Life cycle[36]

The malarial parasite passes its life cycle in two different host, mosquito and man.

Female Anopheles mosquito: Definitive host

Man: Intermediate host.

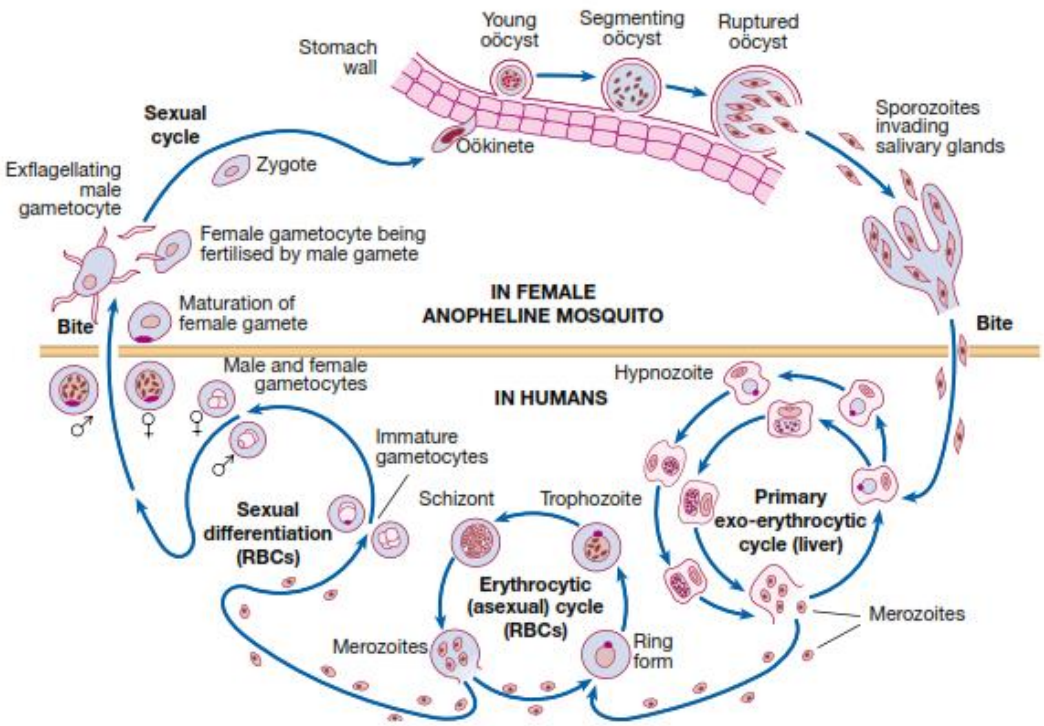


Figure 3. Life cycle of malarial parasite[37]

In mosquito

Sporogony, the sexual cycle, begins when a female mosquito of the genus *Anopheles* ingests circulating male and female gametocytes while feeding on a malaria infected man. In the gut of the mosquito, the gametocytes mature and effect fertilization. The resulting zygote penetrates the mosquito's gut wall, lodges beneath the basement membrane, and vacuolates to form an oocyst. Within this structure, thousands of sporozoites are formed. The enlarging cyst eventually ruptures, releasing the sporozoites into the body cavity of the mosquito. Some penetrate the salivary glands, rendering the mosquito infectious for humans. The time required for the completion of the cycle in mosquitoes varies from 1 to 3 weeks, depending on the species of insect and parasite as well as on the ambient temperature and humidity.

In human

Schizogony, the asexual cycle, occurs in the human and begins when the infected *Anopheles* mosquito bites an individual. Sporozoites from the mosquito's salivary glands are injected into the human's subcutaneous capillaries and circulate in the peripheral blood. Within 1 hour they attach to the liver cells (hepatocytes) and invade them, a process thought to be mediated by a ligand present in the sporozoites' outer protein coat (circumsporozoite protein). In *P vivax* and *P ovale* infections, some of the sporozoites enter a dormant state immediately after cell invasion. The remaining sporozoites initiate exoerythrocytic schizogony, each producing about 2000 to 40,000 daughter cells

(merozoites). One to two weeks later, the infected hepatocytes rupture, releasing merozoites into the general circulation.

The erythrocytic phase of malaria starts with the attachment of a released hepatic merozoite to a specific receptor on the RBC surface. After attachment, the merozoite invades the cell membrane and is slowly endocytosed. The intracellular parasite initially appears as a ring-shaped trophozoite, which enlarges and becomes more active and irregular in outline. Within a few hours, nuclear division occurs, producing the multinucleated schizont. Cytoplasm eventually condenses around each nucleus of the schizont to form an intraerythrocytic cluster of 6 to 24 merozoite daughter cells. About 48 hours (*P vivax, P ovale*, and *P falciparum*) to 72hours (*P malariae*) after initial invasion, infected erythrocytes rupture, releasing the merozoites and producing the initial clinical manifestations of disease. These newly released daughter cells invade other RBCs, where most repeat the asexual cycle while few are transformed into sexual forms or gametocytes. These latter forms do not produce RBC lysis and continue to circulate in the peripheral vasculature until ingested by anopheles mosquito. The recurring asexual cycles continue, involving an ever-increasing number of erythrocytes until finally the development of host immunity brings the erythrocytic cycle to a close. The dormant hepatic sporozoites of *P vivax* and *P ovale* survive the host's immunologic attack and may resume intrahepatic multiplication after a latent period of months to years. This leads to a second release of hepatic merozoites and the initiation of another erythrocytic cycle, a phenomenon known as relapse.

Infection pattern and immunity

When the hepatic schizont rupture, they liberate approximately 10^5-10^6 merozoites, i.e. the product of 5-100 successful sporozoites into circulation. These invade young red cells immediately.

In non immune, the multiplication rate exceeds 10 fold and often reaches 20 fold per cycle. On an average parasites are detectable in the blood on the 11^{th} day after sporozoite inoculation. At this stage the host may be asymptomatic or may have vague non specific symptoms of malaise, headache, myalgia, weakness or anorexia.[38]

On an average, fever begins 2 days later than parasitemia, but in some patients fever precedes parasitemia.

Factors which limit parasite multiplication are:

1. Specific and non specific immune defenses by host.
2. High fever which damages parasite merozoites.
3. Lack of young RBC's —hence iron supplementation may help the diseases process.

The classical description of malaria symptomatology was derived largely from detailed clinical observations made in the late 19^{th} and early 20^{th} centuries, the experience with artificial infections in early chemo therapy studies, and the use of malaria therapy in the treatment of neurosyphilis.[39]

It was apparent from clinical observation and animal studies that some strains of P. falciparum were more virulent than others.[40] The virulence factor probably include - multiplication capacity, cytoadherence and rosetting ability, the potential to induce cytokine release, antigenicity and anti malarial drug resistance.

In P. falciparum the microscopist can see only the first half of the asexual life cycle. In the second half the parasitized cells are sequestered. As a consequence there may be large discrepancies between the number of parasites in the peripheral (circulating) blood and the number of parasites in the body (the actual parasite burden) .[41, 42]

This has often puzzled and misled the clinicians. Some patients appear to tolerate high parasitemia with little adverse effects, where as others die with low parasite count. The clue to the discrepancy lies both in the immune status of the host and in the stage of development of parasites on the peripheral smear.[43]

A predominance of more mature parasite indicates that a greater proportion are sequestered and carries a worse prognosis as compared to a predominance of younger forms. In synchronous P. falciparum infection the peripheral blood parasite numbers fall at the time of sequestration and rise abruptly at the time of merogony (when a predominance of tiny rings are seen).[43]

The other explanation for the ability to tolerate high parasitemia without apparent adverse effects relates to the development of antitoxic immunity.[44]

The host adopts to repeated infection by producing less cytokines for a given quantum of parasites. Eventually a stage is reached where infections are asymptomatic. This is called premunition (partial immunity).

Protective antibodies inhibit parasite expansion through co-operation with the monocyte-macrophage series by binding to parasitised erythrocytes and then activating the Fc receptors.[45]

Non specific effector mechanisms include the activation of phagocytic cells (including neutrophils) to release toxic oxygen species and nitric oxide, both of which are parasiticidal.[46, 47]

The reaction of these oxygen intermediates with lipoproteins produces lipid peroxides. These are more stable cytotoxic molecules and are unaffected by antioxidants.

There is also augmentation of splenic clearance function - both filtration and Fc receptor mediated phagocytosis are increased.[48, 49]

The parasite's proteins expressed on the red blood cell's surface undergo antigenic variation and this is probably instrumental in avoiding complete immune clearance and sustaining the infection.[50, 51]

The monocyte - macro phage series appears to be the most important immune effector cells in the direct attack on parasitised erythrocytes and merozoites.

Following natural infection there is transient humoral response to sporozoites antigens. Sporozoites antibodies decline with a half - life of 3- 4 weeks. The role of a cytotoxic T- cell immune response to the preerythrocytic liver stages in humans is not known. [52, 53]

Strain specific immunity to the asexual stage parasites develops slowly during natural course of the disease but does provide good protection against rechallenge.

Infusion of hyper immune serum to patients with acute malaria can reduce or eliminate parasitemia, mainly through opsonization, phagocytic cell activation and cytotoxicity and augmentation of infected ring form erythrocyte clearance.[54]

Immune serum also reduces parasite multiplication by agglutinating merozoites. It is of interest that malaria does not seem to be worsened by the acquired immune deficiency syndrome (AIDS).[55]

Pathophysiology

The pathophysiology of malaria results from:

1. Destruction of erythrocytes.
2. Liberation of parasite and erythrocyte material into the circulation.
3. The host reaction to these events.
4. Sequestration of vital organs microcirculation by parasite and interfering with micro circulatory flow and host metabolism.

Malaria parasites release cytokines during schizont rupture.[56] A glycolipid material with many of the properties of bacterial endotoxin is released on schizont rupture.[57]

This material appears to be associated with the glycosyl phosphatidyl inositol anchor which covalently links proteins including the malaria parasite surface antigens to the cell membrane lipid bilayer.[58] The parasite products like endotoxin induce activation of the cytokine cascade.

Cells of the macrophage - monocyte series and endothelium are stimulated to release cytokines. Initially tumor necrosis factor (TNF) and interleukin - 1 (IL-1) are produced and these in turn induce release of other pro-inflammatory cytokines including IL-6 then IL-8.[57]

Cytokines are responsible for many of the symptoms and signs of the infection particularly fever and malaise. Plasma concentration of cytokines are elevated, measured by ELISA. There is a positive correlation between cytokine levels and prognosis in severe falciparum malaria. Whether this is a cause or an effect of severe malaria is yet to be decided.[59]

Sequestration

The process where by erythrocytes containing mature forms of P. falciparum adhere to microvascular endothelium (cytoadherence) and thus disappear from the circulation is referred to as sequestration.[60] Sequestration occurs predominantly in the venules of vital organs - greatest in the white matter

of brain and prominent in the heart, liver, kidneys, intestines and adipose tissue and least in the skin.[61]

Cytoadherence

Cytoadherence and the related phenomenon of rosetting leads to microcirculatory obstruction leading to reduced oxygen and substrate supply resulting in anaerobic glycolysis and lactic acidosis.

Cytoadherence is mediated by a family of strain specific high molecular weight proteins derived from parasite termed P. falciparum erythrocyte membrane protein 1 or $PFEMP_1$.[62]

This protein is anchored on the surface of RBC causes knobs on the surface of the red cell and these are attached to vascular endothelium. A protein similar to $PFEMP_1$ called sequestrin has recently been identified on the surface of infected RBC.[63]

Cytoadherence is also mediated by altered red cell membrane components and may be modulated by spleen.[64]

Parasitised RBCs do not cytoadhere in splenectomised monkeys, occasionally patients who have had splenectomy and developed falciparum malaria, in some of them cytoadherence does not occur and all stages of the parasite are seen in the peripheral blood smears.[65]

In presence of a low pH of <7 and high calcium, CD36 - the leucocyte differentiation antigen protein which is present on vascular endothelium (except

brain endothelium) and monocyte-macrophages favors cytoadherence. The inter cellular adhesion molecule (ICAM) will also bind parasitized erythrocytes. This is up regulated by cytokines mainly TNF i.e. cytokine release enhance cytoadherence mainly in cerebral endothelium.[66] The relative importance of the various potential vascular ligands in the pathophysiology of severe P. Falciparum malaria and precise role of spleen remains to be determined.[67]

As there is considerable variability in the cytoadherence determinants in both parasite and the host, severe malaria and the pattern of organ involvement may reflect the result of a particular host- parasite combination.

Rosetting

Erythrocytes containing mature parasites also adhere to uninfected erythrocytes. This process leads to the formation of "Rosettes".[68]

Rosetting shares some characteristics of cytoadherence. It occurs mainly at the middle of the asexual life cycle and it is trypsin sensitive. Unlike cytoadherence, rosetting is inhibited by certain heparin sub fractions and calcium chelators. Rosetting in P. falciparum is associated with cerebral malaria and increased cytoadherence with other vital organ dysfunction.[69]

It has been suggested that rosetting might encourage cytoadherence by reducing flow (shear rate) which would enhance anaerobic glycolysis, reduce pH and facilitate adherence of infected RBC's to venular endothelium.[72] The mechanical obstruction or static hindrance would be compounded by the lack of deformability of the adherent and circulating parasitised red cells.

As the parasite mature inside the erythrocyte, the normally flexible biconcave disc becomes progressively more spherical and rigid.[70] The reduction in deformability results from reduced membrane fluidity, increasing sphericity and the enlarging and relatively rigid intra erythrocytic parasite.[69]

Severe malaria e.g., cerebral malaria, specific immune mediated damage is unlikely as there is no pathological evidence for vasculitis with a cellular infiltrate in or around the cerebral vessels.

Although some glomerular abnormalities have been noted in fatal malaria, the clinical and pathological findings indicate acute tubular necrosis and not glomerular nephritis as the cause of renal dysfunction.[69]

The pathogenesis of pulmonary oedema/ARDS is uncertain. Recent evidence suggest a high permeability oedema secondary to microvascular dysfunction. The endothelial cells show marked cytoplasmic swelling, dilated rough endoplasmic reticulum and swollen mitochondria. Macrophages containing phagocytized malaria pigment filled capillary lumens and infiltrated the interstitium. TNF and interleukin - 1 have been attributed to this.[71, 72]

Acute malaria infections are associated with malaria antigen specific unresponsiveness. This selective paresis is one of the factors contributing to the slow development of an effective and specific immune response in malaria.[73]

Acute malaria is characterized by non specific polyclonal B - cell activation. There is a reduction in circulating T cells with an increase in the δ/γ T - cell subset but other T-cell proportions are usually normal.[74]

Although residents of hyper endemic or holoendemic malarious areas have hyper gamma globulinemia most of this antibody is not directed against malaria antigens.

In non immune individuals, the acute anti body response to infection often comprises IgM or IgG_2 isotypes which are unable to arm cytotoxic cells and thus kill asexual malaria parasites.[75]

These observations suggest immunological basis with specific cellular immune response. In severe malaria there is evidence of a broader immune suppression with defects in monocyte and neutrophil chemotaxis, reduced monocytic phagocytic function and a tendency to bacterial super infection.[75]

Clinical features

The incubation period (time from sporozoite inoculation to the onset of fever) ranges from 9-40 days. Incubation period of Falciparum is 12 days, Vivax is of 14 days, Ovale 17 days and Malariae 28 days. It can be prolonged by partial immunity and chemoprophylaxis.[76, 77]

The clinical features of malaria are determined by the immune status of the host which, in turn, is determined by previous exposure to malaria.

Uncomplicated malaria

The clinical picture of uncomplicated malaria is common to all the four species.

- Initial symptoms – are nonspecific including the lack of a sense of well-being, headache, fatigue, anorexia, malaise, irritability, myalgia, abdominal discomfort and mild fever.
- Typical malarial paroxysms consisting of fever spike, chills and rigor occurring at regular intervals are uncommon, particularly in children <5yrs age. The classical malarial paroxysms consists of 3 stages namely – Cold stage, Hot stage and the Sweating stage.[76,77]

Cold stage

It is characterized by sudden rise of temperature, feeling of intense cold (chills), prompting the need for warmth or cover, and shivering with or without rigors. Usually the rigors last 10-30 mins but may last up to 90 mins.

Hot stage

The patient feels hot and fever becomes high grade. There is severe headache, myalgia, vomiting, tachypnea, palpitation, delirium and prostration. The hot stage lasts for 2-6 hr.

Sweating Stage

It characterized by drenching sweats and rapid fall in temperature, usually it takes 2-3 hrs.

The entire paroxysms may last 8-12 hours, in between the paroxysms the patient appears well.

Signs

Includes mainly anemia and a palpable spleen (usually towards end of the week in non-immune individuals). Slight enlargement of the liver is also common, particularly in young children, which is usually soft and tender. Mild jaundice is common in adults (usually resolves over 1-3 weeks).[77]

Severe and Complicated Malaria

Almost all severe morbidity and mortality in malaria is caused by P. falciparum. Appropriately treated, uncomplicated falciparum malaria carries a mortality rate of approximately 0.1%.[77] The manifestation of severe and complicated malaria include,

Major features [77, 78]

i) Cerebral malaria

ii) Severe anemia (Hb < 5gms% or HCT < 15%)

iii) Acidosis

iv) Hypotension / Shock

v) Renal Failure

vi) Hypoglycemia

vii) Pulmonary edema / ARDS

viii) Bleeding / DIC

ix) Repeated generalized convulsion (>2 /day)

x) Hemoglobinuria

Minor features [77,78]

i) Impaired consciousness

ii) Extreme weakness

iii) Hyperparasitemia (parasite count > 2,50,000 / µl or > 5%)

iv) Jaundice detected clinically or serum bilirubin > 3 mg/dl.

v) Hyperpyrexia (rectal temperature > 40°C)

Severe malaria

One or more of the above features (Major or Minor) in the presence of asexual parasitemia.[77,78]

In severe malaria there is often evidence of multiple organ dysfunction and more than one of the above criteria are fulfilled.

Cerebral malaria

This is the most dreaded complication of P.falciparum infection. Very rarely, P.vivax infection has been reported to cause cerebral malaria.

It is defined as unarousable coma i.e. there is no response to a painful stimulus or altered sensorium. The onset of coma may be sudden often following a generalized seizure or gradual with initial drowsiness, confusion, disorientation, delirium or agitation followed by unconsciousness. [76-78] On examination the patient is febrile, unarousable with or without signs of meningitis like neck rigidity. Anemia, Jaundice and hepatosplenomegaly will be there.

The clinical features are usually of symmetrical encephalopathy. Focal signs are unusual. Papilledema is rare (<1%) but retinal hemorrhages are seen in 15% of cases. The hemorrhages are often flame shaped and may have a pale centre resembling the Roth spots. Bruxism (forced jaw closure with repetitive spontaneous teeth grinding), brisk jaw jerk and a pout reflex may be present. Other frontal release signs are unusual. Cranial nerves abnormalities are rare. Deep tendon reflexes are brisk and absent abdominal reflexes. Patient may exhibit phasic increase in tone with extensor posturing of the decorticate (arm flexed and legs extended) or decerebrate (arms & legs extended) type rigidity.[76-78]

Untreated malaria is uniformly fatal, and 20% mortality in treated patients and up to 50% mortality in pregnancy has been reported[60]

Bad prognostic signs are: [79]

- Longer history of coma and delayed diagnosis.
- Absence of corneal reflex.
- Decerebrate rigidity.
- Extreme agitation.
- Signs of bleeding (DIC).
- Sustained hyperventilation due to metabolic acidosis (chest infection or ARDS)
- Generalized or recurrent seizures.

Features indicating a poor prognosis on admission

Clinical - agitation, hyperventilation, hypothermia (<36.5°C), hypotension (shock) sustained hyperthermia (>39 °C), bleeding, severe anemia (<15%), deep coma, convulsion, anuria, deep jaundice and signs of hepatocellular failure.[80,81]

Laboratory[80,81]

Hypoglycemia	< 40 mg% of RBS
Hyperlactatemia	> 5 mmol/L
Severe acidosis Arterial pH	< 7.3, HCO_3< 15 mmol/L
Serum creatinine	> 3 mg% (more than 265 µmol/L)
Total bilirubin	> 3 mg%
Liver enzymes	> 3 times the normal values
Muscle enzyme	Increased CPK and myglobin
Uric acid	> 32 mg%

Hematological

Leukocytosis	> 12,000 cells /µL
Severe anemia	PCV < 15%
Coagulopathy	Platelets < 50,000/µL
Prothrombin time	>3 seconds
Partial thromboplastin time	Prolonged
Fibrinogen level	< 200 mg%
Parasitic index	Hyperparasitemia >1,00,000/µL - increased mortality. >5, 00,000 /µL - high mortality. > 20% of parasites are pigment containing trophozoites and schizonts. >5% of neutrophils contain visible pigment.

Pathology

In falciparum malaria the microvasculature of the vital organs is packed with erythrocytes containing mature form of the parasite. There is abundant intra and extra erythrocytic pigment and organs such as liver, spleen and placenta may be grey- black in colour. Sequestration is not uniformly distributed. It tends to be most in the brain and heart followed by the gut, kidney, adipose tissue, liver, lungs and least of all in bone marrow and skin.[82]

Brain

The brain is swollen with multiple small petechial hemorrhages throughout the white matter. Hemorrhages are unusual in the grey matter. No evidence of tentorial or foramen magnum herniation is seen. Nearly every capillary and venule is packed with erythrocytes containing mature forms of the parasite (where as these are rarely seen in peripheral smear). The sequestration is particularly prominent in the white matter although the tissue is much less vascular than grey matter.[60]

The degree of cerebral sequestration and the intensity of erythrocyte packing is greater in cerebral malaria than in fatal malaria in which the patient is not comatose. In the white matter accumulation of glial cells are seen surrounding hemorrhagic foci (Durck's granuloma) where vessels appear to have been occluded by a mass of parasitized cells and then ruptured.

At the ultra-structural level, erythrocytes are seen to be packed closely together and the infected red cells are adherent to the vascular endothelium by attachment of knob like surface projections to the endothelial surface.

Occasional fibrin strands are seen but there is a striking absence of platelets and no evidence for leucocyte aggregation i.e. there is no evidence of thrombus formation or vasculitis.[61]

Heart and lungs

Despite intense sequestration in the myocardial vasculature the heart is remarkably normal. In anemic patients it may be pale and dilated. Evidence of pulmonary oedema with hyaline membrane formation suggesting leakage of proteinaceous fluid may be seen. Moderate sequestration and leucocytes aggregation may be seen.[83]

Liver and spleen

Liver is enlarged and may be black due to malaria pigment. There is congestion of the centrilobular capillaries with sinusoidal dilatation and kupffer cell hyperplasia. Sequestration of parasitized erythrocytes is associated with variable cloudy swelling of the hepatocytes and perivenous ischemic changes and sometime centrizonal necrosis.

Spleen is dark from malaria pigment, enlarged, soft and friable. It is full of erythrocytes containing mature and immature parasites. There is evidence of reticular hyperplasia and architectural reorganisation.[84] The soft and acutely

enlarged spleen of acute lethal infections contrasts with the hard fibrous enlargement associated with repeated malaria.

Kidney

Kidneys are slightly swollen. Tubular abnormalities are consistent with ischemia. There is sequestration in glomerular capillaries and sometimes mesangial and endothelial cell proliferative changes are seen. Immuno-fluorescent studies show immunoglobulin deposition in the glomerular capillary basement membranes.[85]

Alimentary tract

Upper GI bleeding from erosions may be seen. Intense sequestration in the gut and the visceral ischemia explain the acute abdominal pain.[85] Despite this drug absorption is often remarkably normal.

Bone Marrow

Dyserythropoietic changes are prominent. Bone marrow macrophages contain pigment erythrophagocytosis and malaria parasites may be seen.[86]

Placenta

May be black due to malaria pigment and a large number of mature parasites are seen although peripheral smear may be negative. There is often trophoblastic thickening, macrophage infiltration and perivillous fibrin deposition.[87]

Consequences

Black Water fever

Black water fever[88,89] is the result of massive intravascular hemolysis and the passage of "coca-cola" coloured urine. Pathogenesis is poorly understood. It occurs in three circumstances;

1. When patients with G6PD deficiency take oxidant drugs irrespective of whether they have malaria or not.
2. Patients with G6PD deficiency having malaria and on quinine therapy.
3. Normal P. Falciparum patients treated with quinine.

G6PD deficient red cells are particularly susceptible to oxidant stress as they are unable to synthesize adequate quantities of NADPH through the pentose shunt pathway. This leads to low intraerythrocytic levels of reduced glutathione and both alterations in the erythrocyte membrane and increased susceptibility to organic peroxides.

Fluid and electrolyte changes

Total body water and extra cellular volume are normal. Plasma renin activity, aldosterone and anti-diuretic hormone concentrations are elevated reflecting an appropriate activation of homeostatic mechanism to maintain adequate circulating volume in the presence of general vasodilatation and a falling hematocrit. Mild hyponatremia and hypochloraemia are common but serum potassium is usually normal.

Algid malaria

The majority of patients with severe malaria remain well perfused and warm but some develop shock with cold extremities. This is known as algid malaria.

This may result from secondary gram negative bacteremia and hypovolemia (due to dehydration and rarely hemorrhage). Patients with severe malaria are vulnerable to bacterial infections due to transient immunosuppression and impaired macrophage function or blockade of the reticulo-endothelial system.

Chronic complications of malaria

Repeated malarial infections can cause chronic complications like tropical splenomegaly syndrome, nephrotic syndrome or quartan malarial nephropathy, Endemic Burkitts lymphoma and endomyocardial fibrosis.

Laboratory diagnosis

Malaria is diagnosed by microscopic examination of the blood for the evidence of parasites. Peripheral smear of capillaries or venous blood are usually tested. In difficult to diagnose situation blood[90] and/or bone marrow smears are useful.[91] Liver or splenic biopsies or after injection of norepinephrine subcutaneously and blood smear examination after half an hour may yield positive results due to splenic contraction.

For routine peripheral smear thick and thin smears are done, thick film for parasite identification and thin film for parasite counting and stage identification. The smears are stained by Leishman or Giemsa or Field's stain.

Density of parasitemia is measured in thick film by counting number of parasites and number of leucocytes in each filed and using - total leucocyte count to number of parasites per cubic mm.[92]

Grading

1 - 10-MP	-	100 fields	+
10-100 –MP	-	100 fields	++
1-10-MP	-	One field	+++
10-l00-MP	-	One field	++++

Intensity of infection is measured in thin films i.e., percentage of RBCs Parasitised /1000 RBC's (10-100%).

Parasite index (PI): Number of parasites per 1000 RBC's

The number of paratised Red cells per 100 RBCs / 1000 should be counted. If there are two parasites in one red cell, this is counted as one. At low parasitemia (<5/1000 on the thin film) the thick film should be counted. The number of parasites per 500 white cells are counted. The figures are corrected by the total red cell and white cell counts to give the number of parasites per unit blood volume (μL).

If parasitemia is high the stage of parasite development should be assessed on the thin film. The proportion of asexual parasites containing visible pigment i.e. mature trophozoites and schizonts should be counted. The presence of pigments in neutrophil and monocytes are also noted. In patients who are already on treatment but the pigment may still be present in leucocytes after clearance of parasitemia, giving an important clue to the diagnosis. Monocytes containing pigment are cleared more slowly than pigment containing in neutrophils.

Other techniques

- Fluorescent dye acridine orange staining of malaria parasites (which contain DNA and RNA) and visualizing them under ultraviolet light microscopic after collecting blood in specialized capillary tubes for testing.
- Detection of monoclonal antibody against P. Falciparum histidine rich protein 2 by a rapid and simple stick test.
- Quantitative buffy coat.

Quantitative buffy coat

The QBC Test, developed by Becton and Dickenson Inc., is a new method for identifying the malarial parasite in the peripheral blood. It involves staining of the centrifuged and compressed red cell layer with acridine orange and its examination under UV light source. It is fast, easy and claimed to be more sensitive than the traditional thick smear examination.[93]

Method

The QBC tube is a high-precision glass hematocrit tube, pre-coated internally with acridine orange stain and potassium oxalate. It is filled with 55-65 microliters of blood from a finger, ear or heel puncture. A clear plastic closure is then attached. A precisely made cylindrical float, designed to be suspended in the packed red blood cells, is inserted. The tube is centrifuged at 12,000 rpm for 5 minutes. The components of the buffy coat separate according to their densities, forming discrete bands. Because the float occupies 90% of the internal lumen of the tube, the leukocyte and the thrombocyte cell band widths and the top-most area of red cells are enlarged to 10 times normal. The QBC tube is placed on the tube holder and examined using a standard white light microscope equipped with the UV microscope adapter, an epi-illuminated microscope objective. Fluorescing parasites are then observed at the red blood cell/white blood cell interface.

« Platelets

« Lymphocytes/ monocytes

« Granulocytes

« Red Blood Cells

Figure 4. The QBC Tube

The key feature of the method is centrifugation and thereby concentration of the red blood cells in a predictable area of the QBC tube, making detection easy and fast. Red cells containing Plasmodia are less dense than normal ones and concentrate just below the leukocytes, at the top of the erythrocyte column. The float forces all the surrounding red cells into the 40 micron space between its outside circumference and the inside of the tube. Since the parasites contain DNA which takes up the acridine orange stain, they appear as bright specks of light among the non-fluorescing red cells. Virtually all of the parasites found in the 60 microliter of blood can be visualized by rotating the tube under the microscope. A negative test can be reported within one minute and positive result within minutes.

Comparison between peripheral smear and QBC test for detecting malaria

	Peripheral smear	**QBC**
Method	Cumbersome	Easy
Time	Longer, 60 - 120 minutes	Faster, 15 - 30 minutes
Sensitivity	5 parasites/µl in thick film and 200 / µl in thin film	Claimed to be more sensitive, at least as good as a thick film
Specificity	Gold standard	? False positives, artifacts may be reported as positive by not-so-well-trained technicians
Species identification	Accurate, gold standard	Difficult to impossible
Cost	Inexpensive	Costly equipment and consumables
Acceptability	100%	Not so
Availability	Everywhere	Limited
Other	--	Accidentally can detect filarial worms

Blood picture

- Normochromic normocytic anemia.
- Total leucocyte count is normal or increased in severe malaria with occasionally leucoerythroblastic picture.
- Monocytosis with eosinopenia with reactive eosinophilia in the weeks following the acute infection.[94]
- Thrombocytopenia with fibrinogen and fibrin degradation products are increased. A reduction of fibrinogen usually indicate disseminated intravascular coagulation. There is evidence of increased coagulation cascade activity through intrinsic pathway activation with antithrombin III depletion i.e. proportional to disease severity.
- Prothrombin time and partial thromboplastin times are prolonged.[41]
- Polymorphonuclear leucoelastase levels are elevated in severe infection suggesting neutrophil activation.[92]
- The C-reactive protein, α1-acid glycoprotein and fibrinogen levels are raised. While albumin level falls immunoglobulin and cytokine levels are raised.

Blood urea and serum creatinine may be raised with an increased urea to creatinine ratio. Total and conjugated bilirubin, transaminases and alkaline phosphatase levels are usually elevated depending upon the severity. Creatinine phosphokinase, myoglobin and plasma urate levels are raised. Low serum calcium and phosphorous levels are seen. Hypoglycemia is usually evident and profound in patients with quinine therapy. In the absence of therapy there may be

associated elevation of ketones, lactates and alanine levels with hypoinsulinemia. Hyponatremia with normal potassium are seen normally. Bicarbonate levels are reduced and the anion gap widens in proportion to the acidosis. Urine shows hemoglobinuria and traces of albumin.

Cerebrospinal fluid (CSF)

Usually normal but moderately raised proteins and lymphocytes may be seen. The lactate concentration is raised proportional to the disease severity.[79,92]

Lipid profile in malaria infected patients

Malarial infection is associated with life threatening and debilitating conditions such as fever, chills, myalgia, headache, nausea, vomiting and diarrhea. Lipoproteins such as chylomicrons, very low density lipoproteins (VLDL), low density lipoproteins (LDL), high density lipoproteins (HDL) and free fatty acids (FFA) are major lipid components in plasma. Most plasma apolipoproteins, endogenous lipids and lipoproteins have their origin from the liver, which depends on cellular integrity and functionality of the hepatocytes. Under normal physiological conditions, liver ensures homeostasis of lipid and lipoprotein metabolism. Hepatocellular damage often associated with severe and acute P. falciparum infections impairs these processes, leading to alterations in plasma lipid and lipoprotein patterns. Likewise, hyperbilirubinemia, increased plasma levels of aspartate amino transferase (AST) and alanine amino transferase (ALT) activities are strong evidence of gross hepatocytic dysfunction in patients with P. falciparum infection.[95]

Relationship between serum lipid profile and severity of P. falciparum and other parasitic infections in human has drawn the attention of various research authors and has been proposed as a basis for diagnosis and severity of the disease.[95]

Transitory changes in the plasma levels of lipids, cholesterol and triglycerides have been observed since a long time by many authors in different acute infections. Hypocholesterolemia, hypertriglyceridemia and extreme decrease in HDL and LDL fractions were observed in complicated and uncomplicated malaria. The magnitude of these changes seems to be related with severity of malaria. Hyperlipidemia, a hallmark of malarial infection which result in depletion of natural antioxidants and facilitates the production of reactive oxygen species (ROS) which has the ability to react with all biological molecules like lipids, proteins, carbohydrates, DNA etc. and exerts cytotoxic effects on cellular components. Thus increased ROS and impaired antioxidant defense contributes for initiation and progression of micro and macro vascular complications in malaria.[96]

Pathophysiological pathways of lipids during malaria

Lipids are synthesized in the liver, which incidentally happens to be the site where infective malaria sporozoites travel through the bloodstream, invade and take residence. In this asymptomatic 'exo-erythrocytic stage' these cells divide until many mature tissue schizonts are formed, each containing thousands of merozoites. The liver schizonts rupture after days and release these merozoites into the bloodstream, initiating the 'erythrocytic stage'. Within the erythrocyte, a

single merozoite divides into eight to 32 merozoites, which demands a considerable amount of lipids (e g, cholesterol) for their anabolic requirements such as membrane formation. The capacity of *Plasmodium* species to replicate is noteworthy, achieving one of the fastest growth rates among eukaryotic cells. Malaria parasites are intracellular protozoans, auxotrophic and unable to synthesize organic nutrients required for their growth. To ensure their survival and propagation, they must exchange nutrients over the parasitophorous vacuolar membrane (PVM), which surrounds the parasite. This PVM neither acidifies nor fuses with organelles of the endocytic cascade and exocytic pathway and is thus actually completely isolated from the host cell vesicular transport system.[15]

Keeping this in view, the following questions arise. First, what is the relation between malaria parasites and lipid synthesis in the liver - are malaria parasites capable of producing essential lipids themselves or are host lipids required? Second, does the malaria parasite benefit from high or low serum lipids in the host environment.[15]

In order to meet the nutrients to rapidly multiply within hepatocytes, but more importantly, red blood cells, malaria parasites must scavenge host cell nutrients as they cannot synthesize. *Plasmodium* species cannot synthesize cholesterol by itself, similar to other intracellular pathogens. These pathogens most often possess the ability to access this lipid from the exogenous or endogenous pathway of the host. Also, no biological pathway involved in sterol production can be demonstrated in *Plasmodium* species genome databases. It must be said however, that, morphological data suggest that the parasitophorous vacuole of malaria liver forms does contain sterols.[15]

Recent findings demonstrate that the *Plasmodium* genome includes gene-encoding enzymes for phospholipids metabolism, allowing *de novo* synthesis of phosphatidylcholine via the Kennedy Pathway (*de novo* synthesis of phosphatidylethanolamine and phosphatidylcholine) and necessitating only the uptake of the small choline molecule. This is important, because these two account for more than 50% of the total phospholipids in eukaryotic membranes and thus play a major role in the structure and function of the membranes. Moreover, the genome of *P. falciparum* has genes similar to those encoding for the type II fatty acid synthesis pathway in humans. The type II fatty acid synthetic pathway is the principal route for the production of membrane phospholipidacyl chains. These particular genes are embedded within the apicoplast, and aid the production of fatty acids, some of which are unique for *Plasmodium* species. Therefore, *Plasmodium* species might be able to meet several of its lipid needs from its own biological pathways, even if specific extracellular lipids are necessary for *in vitro* growth. The presence of cholesterol in apicoplast membranes was shown only recently.[15]

However, the inability of *Plasmodium* to stock up host molecules makes a continuous supply of nutrients to the parasite necessary. Probably, this is one of the reasons that malaria parasites choose hepatocytes, as they have unique metabolic properties and are especially efficient in internalizing transport proteins (e. g, lipoproteins) via membrane receptors and are proficient at metabolizing different compounds (e. g, glucose, lipids etc.) in relatively huge quantities.[15]

A recent study[97] shows that *Plasmodium* divert cholesterol from the hepatocyte cell until the release of merozoites. Removal of plasma lipoproteins *in*

vitro resulted in a 70% reduction of cholesterol content in hepatic merozoites. It was discovered that *Plasmodium* spp. (*P. yoelii* and *P. berghei)* salvage cholesterol that had been internalized by LDL. However, reduced expression of host LDL receptors did not influence liver stage burden. *Plasmodium* is also capable of seizing cholesterol produced by hepatocytes. Pharmacological blockade of host squalene synthase (an enzyme involved in the first step in sterol synthesis) or the down-regulation of the expression of this enzyme by 80% diminished the cholesterol content of merozoites without effect on parasite development. These data suggest that malaria parasites do need sterols for effective replication, but can also adapt to cholesterol-restrictive conditions by using alternative sources in hepatocytes to maintain infectivity.[97]

Another study demonstrated that HDL is essential for the maintenance of *P. falciparum* in *in vitro* culture.[98,99] At relatively low concentrations (0.75 mg/ml protein) HDL is able to aid parasite growth and reinvasion in a serum-free system. In higher concentrations (2.4 mg/ml protein), HDL is toxic to the parasite within infected erythrocytes after invasion, causing abnormal maturation and death of trophozoites.[92]

Late ring stage parasites at a parasitaemia of 2% were cultured with HDL or with phosphate buffer (controls). In the HDL-treated group, unfit parasites developed with reduced size, irregular shape, increasing stain density, and haemozoin (malaria pigment) outside the food vacuole.[92]

Both host cholesterol synthesized in the endoplasmic reticulum as well as LDL-derived cholesterol are co-transported to the parasitophorous vacuole. Most

probably, compensatory activity of the endogenous and exogenous pathways to provide cholesterol to the parasite exists. Furthermore, the parasite could also cause a hypocholesterolaemia in malaria because it utilizes another pathway, that of receptor-mediated endocytosis[100] where cholesterol is extracted from the blood.

Besides the role of lipids in the proliferation and metabolism of the parasite, host lipids have also been implicated in the formation of haemozoin *in vivo*.[15]

Earlier, it has been shown that linoleic acid (a polyunsaturated fatty acid) may be necessary for the dimerization of ferriprotoporphyrin IX (a toxic compound released after the digestion of hemoglobin), the initial step in the production of haemozoin. Haemozoin is the end product of the plasmodial detoxification of free haem that is produced by haemoglobin degradation. Historically, it was thought that haemozoin was an inert waste product of the malaria parasite.[15]

However, recent research resulted in the recognition of the importance of haemozoin in different aspects of malaria.[101] Haem crystalization is the target of the widely used anti-malarial aminoquinoline drugs.[15] Moreover, not only does the haemozoin production require host lipids, but it appears also that the inhibition of host monocyte functions, one of the eminent immune-modulating haemozoin effects, is caused by hydroxyl fatty acids, generated by *Plasmodium* species in large amounts in the human hosts. The lipid hypothesis postulates that haemozoin formation occurs most rapidly at lipid-water interfaces. In the past

three years, convincing evidence is emerging in favour of the lipid model. The lipid environments in a parasitized erythrocyte using Nile Red stain (a lipophilic stain), were characterized.[15]

Neutral lipids associated with the digestive vacuole of the parasite were observed. These were composed of di- and triacylglycerol's (triglycerides); possibly storage organelles for lipid intermediates produced during the degradation of phospholipids in the food vacuole. Mono-, di- and triacylglycerol heterogeneous mixtures promote haemozoin formation, implying that these neutral lipids are involved in haem detoxification.[15]

It was demonstrated that triglycerides are a major lipid portion stored in lipid droplets in the late trophozoite and schizont stage of *P. falciparum*. Besides haem detoxification, it may be utilized to store acyl groups for phospholipid synthesis, glycosyl phosphatidyl inositol (a glycolipid) synthesis, and possibly for beta-oxidation (the process by which fatty acid molecules are broken down in the mitochondria to generate acetyl-coA).[102]

Another study demonstrated that in the unrelated haemozoin-forming organisms *Schistosoma mansoni*, and in the kissing bug, *Rhodnius prolixus* (triatomine vector of Chagas disease), haemozoin formation occurs inside lipid droplet-like particles or in close association to phospholipid membranes (both hydrophobic environments).[103]

It has been also been reported that the intracellular mechanism of molecular initiation of haemozoin formation occurs within neutral lipid predominant nanospheres, which envelop haemozoin inside *P. falciparum*

digestive vacuoles. It was suggested that haemozoin is formed at the interface between the aqueous medium of the food vacuole and the lipid nanospheres.[15]

Another study confirmed these findings, as molecular dynamic simulation showed that a precursor haemozoin dimer forms spontaneously in the absence of the competing hydrogen bonds of water, demonstrating that this substance probably self-assembles near a lipid/water interface *in vivo*. Probably, haemozoin nucleation occurs at the digestive vacuole inner membrane, with crystallizations occurring in the aqueous rather than lipid phase, as indicated by cryogenic soft X-ray tomography.[104-107]

Thus, lipids mediate synthetic haemozoin formation very efficiently. Further weight is added to this lipid hypothesis by another recent study that demonstrated that haemozoin-associated neutral lipids alone are capable of mediating haemozoin formation at adequate rates under physiologically realistic conditions of ion concentrations to account for haemozoin formation.[108]

The combination of these recent insights makes a compelling case for the theory that lipids drive haemozoin assembly.[15]

The time for malaria patients to recover from the serum lipid profile alterations varied widely across studies. Studies[109-114] reported measurements after “day 0” (admission, before anti-malarial treatment). In most studies, lipid parameters resolved slowly; in one study levels of cholesterol, HDL and LDL were significantly lower in the malaria patients than in the control group at one month after treatment. Most lipid levels had increased at six months while triglyceride levels continued to be lower than normal parameters.[114] These

findings contrast with findings from a study in travelers with malaria; both LDL and plasma triglyceride concentrations were normalized at follow up after 2 weeks.[112] A study with 152 *P. vivax* patients showed that TC, ester and free cholesterol reached normal levels in ten days.[115]

A first systematic review and meta-analysis of the impact of malaria on common lipid profile parameters confirmed previous studies that characteristic serum lipid profile changes occur during malaria. Low serum TC, a low HDL, a low LDL during malaria are described as compared to reference values, in healthy and symptomatic controls. Triglycerides were raised during malaria, but this was statistically not significant when compared to symptomatic controls. The conclusion is supported by a similar size and direction of lipid profile changes noted in the records not included in the quantitative synthesis. For IDL cholesterol, VLDL and apolipoproteins, no robust alterations could be observed due to complete absence and paucity, respectively, of studies that measured these laboratory parameters.[15]

The major difficulty regarding the clinically observed serum lipid profile changes is whether they are not only characteristic but truly specific for malaria, rather than a general observed phenomenon that can also be seen in other conditions, particularly (infectious) diseases. Moreover, the association between serum lipid profile changes and malaria is not definitive evidence for the direction of causality, since the existence of confounders, for example, ethnicity, socio-economic status, life-style, food habitats, other infections or diseases, etc., cannot be ruled out and was not corrected for in most of the included studies in that review.[15]

The meta-analysis that included comparisons between malaria patients and symptomatic controls suggests that the observed lipid profile changes are indeed specific for malaria. TC, HDL and LDL concentrations were lower in malaria and other febrile diseases compared to healthy controls, however, the decline was more pronounced and statistically significant during malaria. If these lipid profile changes are characteristic for malaria, one could expect more pronounced lipid alterations in severe malaria compared to uncomplicated malaria; this is confirmed by three studies.[111,116,117] Biological mechanisms of lipid profile changes may be partly host-related, i.e., related to an acute phase reaction or parasite-related or a combination of these two.[15]

Host-related lipid profile changes

Transient plasma lipid profile changes are not only observed during malaria, but also in other acute diseases. Typically, HDL and LDL cholesterol levels are slightly reduced, and VLDL levels may be increased. Several researchers demonstrated low cholesterol levels in acute conditions such as surgical trauma, malignancy, burns and ischemic heart disease. Hence the changes in plasma lipoproteins appear to form part of the acute-phase reaction and can, at least partially, be ascribed to extravasation due to increased capillary permeability. In addition, a decrease in TC and triglycerides has been reported in patients admitted with an acute infection.[15]

In a study with critically ill patients, the mean HDL level was significantly lower in patients with an infection compared to patients without

infection. The TC levels seemed to be slightly lower and triglycerides higher in infected patients, but these differences were not statistically significant.[118]

Various forms of lipid disorders have been associated with acute and chronic infectious diseases of different etiologies: bacterial, viral and parasitological. In human immunodeficiency virus (HIV) infection an increase in the level of triglycerides and decrease in levels of cholesterol, HDL and LDL have been observed. Moreover, the treatment of HIV with highly active antiretroviral therapy (HAART) can cause a more atherogenic lipid profile by increased TC, LDL-c and triglycerides. Hypertriglyceridemia has been described in several diseases with haemophagocytosis.[15]

Hypocholesterolaemia has also been described in various haematological diseases, including thalassaemia major, thalassaemia intermedia, sickle cell disease, glucose-6-phosphate dehydrogenase (G6PD) deficiency, spherocytosis, and aplastic anemia. It can also accompany anemia with high erythropoietic activity. The pathophysiology of the lowered cholesterol levels in these diseases remains obscure, although several mechanisms have been proposed, including the dilution of serum due to anemia; increased cholesterol need associated with erythroid hyperplasia; macrophage activation with the release of cytokines; increased cholesterol uptake by the reticulo-endothelial system and liver injury secondary to iron overload.[15]

A review reported that, serum total cholesterol was measured in 36 of 42 included studies and 83% (30/36 studies) reported a hypocholesterolaemia in

patients with malaria or a significantly lowered total cholesterol level compared to the control group.[15]

Two studies[96,119] reported a raised cholesterol, two studies showed no significant differences with the control group[12,121] and one study was inconclusive.[122] The mean difference for cholesterol in malaria patients versus healthy controls was reported as 1.09 mmol/l or 42.15 mg/dl (95% CI 0.74-1.44 mmol/l), $I^2 = 98\%$, $Z = 6.14$ $P < 0.00001$. Further, the mean difference for cholesterol in malaria patients *versus* symptomatic controls was 0.79 mmol/l or 30.55 mg/dl (95% CI 0.13-1.45 mmol/l), $I^2 = 90\%$, $Z = 2.34$, $P = 0.02$. Thus, cholesterol was significantly decreased during malaria. For severe malaria, a separate analysis including three studies[111,116,123] showed mean difference for cholesterol in severe malaria patients *versus* healthy controls was 1.60 mmol/l or 61.87 mg/dl (95% CI 0.66-2.54), $I^2 = 99\%$, $Z = 3.33$, $P = 0.0009$.

In a review of the 42 studies, 23 included HDL patients with malaria and 87% (20/23 studies) reported a large decline in HDL-concentrations. The mean difference for HDL in malaria patients *versus* healthy controls was 0.32 mmol/l or 12.37 mg/dl (95% CI 0.02-0.63 mmol/l), $I^2 = 99\%$, $Z = 2.08$, $P = 0.04$. Also, the mean difference for HDL in malaria patients *versus* symptomatic controls was 0.39 mmol/l or 15.08 mg/dl (95% CI 0.07-0.72 mmol/l), $I^2 = 85\%$, $Z = 2.39$, $P = 0.02$.[15] Two studies[116,123] showed a significant larger decline in HDL in severe malaria compared to uncomplicated malaria. Thus, HDL is significantly lower in malaria.

A review[15] reported that 16 studies assessed LDL in patients with malaria and 81% (13/16 studies) reported a lower LDL-c concentration in malaria patients. The mean difference for LDL in malaria patients *versus* healthy controls was found to be 0.82 mmol/l or 31.71 mg/dl (95% CI 0.24-1.39 mmol/l), I^2 = 97%, Z = 2.79, P = 0.005. Only one study compared LDL in malaria patients with symptomatic controls and found a difference of 1.67 mmol/l or 64.58 mg/dl (95% CI 1.44-1.90 mmol/l), P < 0.01.[114] Two studies[116,123] showed a significant larger decline in LDL in patients with severe malaria compared to patients with uncomplicated malaria. Thus, LDL is significantly lower in malaria.

A review[15] reported that, 78% (18/23 studies) reported a hypertriglyceridemia and/or a significantly higher mean triglyceride plasma concentration in malaria patients compared to controls. The mean difference for triglycerides in malaria patients *versus* healthy controls was 0.25 mmol/l or 22.14 mg/dl (95% CI 0.12-0.37 mmol/l), I^2 = 82%, Z = 3.79, P = 0.0002 and the mean difference for triglycerides in malaria patients *versus* symptomatic controls was 0.42 mmol/l or 37.20 mg/dl (95% CI 0.46-1.31 mmol/l), I^2 = 95%, Z = 0.94, P = 0.35. Thus, triglycerides are significantly higher in malaria patients compared to healthy controls, but these differences become non-significant when compared to symptomatic controls. In patients with severe malaria triglyceride levels were found to be higher compared to triglyceride levels in patients with uncomplicated malaria.[111,116]

Studies have shown variation in common lipid parameters in malaria infected patients.

A study performed by Krishna AP et al[96] to assess the lipid parameters in malaria infected patients found that there is significant decrease in HDL cholesterol and LDL cholesterol and increase in triglycerides.

A study performed by Sibmoooh N et al,[124] suggested that alteration of lipid metabolism during acute infection may result from acute phase response. The acute phase response is associated with changes in lipid metabolism including a moderate increase in serum triglycerides and VLDL, but decrease in HDL and LDL.

Another study, performed by Sin C, et al.[125] noted that there is significant decrease in serum HDL in malaria infected patients and concluded that serum HDL may be valuable information in the diagnosis of the malaria.

Mohanty S, et al[111] conducted a case control study to study malaria induced changes in plasma lipids and found that there was decrease in LDL and HDL in cases when compared to controls.

Chapter 4

Methodology

METHODOLOGY

The present study was conducted in the Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum from January 2013 to December 2013.

Study design

The study design was a cross sectional study.

Study period and duration

This study was conducted from January 2013 to December 2013 for a period of one year.

Place

The present study was conducted in the Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum a teaching hospital attached to Jawaharlal Nehru Medical College, KLE University, Belgaum.

Source of Data

The study comprised of adult patients presenting with malaria infection.

Sample size

A total of 50 adult patients presenting with malaria infection were selected for the study.

Sampling procedure

The sample size was determined considering 80% of the average past three years hospital statistics.

Selection criteria

Inclusion

- Patients age > 12 years
- Patients with symptoms and signs of malaria and smear positive for malaria and/or positive QBC test for malaria.

Exclusion

- Patient on lipid lowering drugs.

Ethical clearance

Prior to the commencement, ethical clearance was obtained from Institutional Ethics Committee, Jawaharlal Nehru Medical College, Belgaum.

Informed Consent

The patients fulfilling selection criteria were explained about the nature of the study and a written informed consent was obtained (Annexure I).

Method of collection of data

Patients were interviewed for demographic data such as age, sex, occupation etc. were noted. Histories of similar complaints in past and current treatment were noted. Patients were subjected to a thorough physical

examination, vitals (pulse rate, temperature, blood pressure and respiratory rate) and other clinical signs and symptoms suggestive of malaria infection were noted. Systemic examination was carried out. These findings were recorded on a predesigned and pretested proforma (Annexure II).

Investigations

The selected patients underwent the following investigations.

- Complete blood count
- Random blood sugar
- Peripheral smear for malarial parasite
- QBC test for malaria
- Fasting lipid profile
    - Cholesterol
    - Low density lipoprotein
    - High density lipoprotein
    - Triglycerides

Procedure for lipid profile estimation

Serum lipid profile estimation was done by a Fully Automated Siemens Dimension Clinical Chemistry System.

Outcome variables

- Total Cholesterol
- Low density lipoprotein

- High density lipoprotein
- Triglycerides

Statistical analysis

The data obtained was coded and entered into Microsoft Excel Worksheet (Annexure III). The categorical data was expressed as rates, ratios and proportions. The continuous data was expressed as mean ± standard deviation (SD) and comparison was done using independent sample‘t’ test. The comparison of more than three mean ± SD was done using one way ANOVA. A probability value (‘p’ value) of less than or equal to 0.050 at 95% confidence interval was considered as statistically significant.

Chapter 5

Results

RESULTS

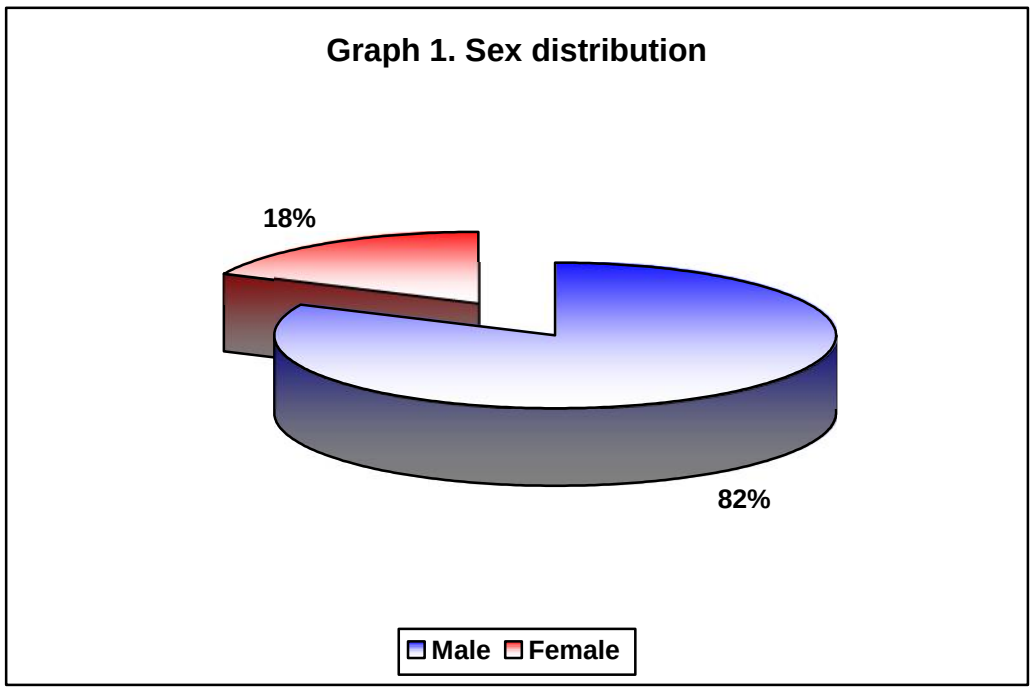
This one year cross sectional study was conducted in the Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum from January 2013 to December 2013.

A total of 50 adult patients presenting with clinical features suggestive of malaria infection were selected for the study.

Data obtained was coded and entered into the Microsoft Excel Spreadsheet. The data was analyzed and the final results and observations were tabulated as below:

Table 1. Sex distribution

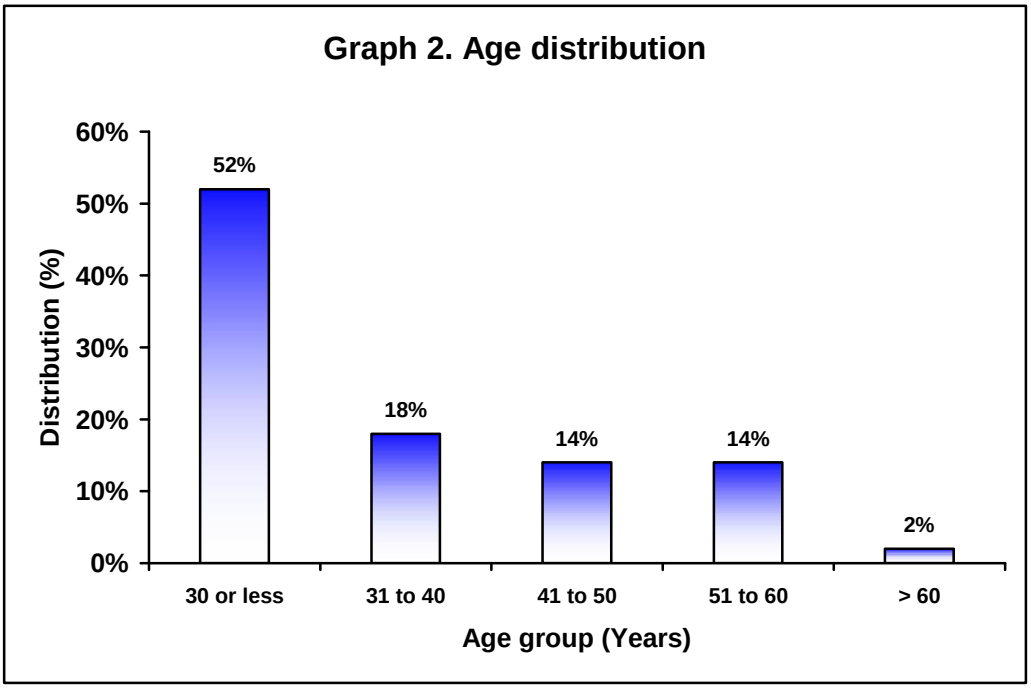
Sex	Distribution (n=50)	
	Number	**Percentage**
Male	41	82.00
Female	9	18.00
Total	**50**	**100.00**



In the present study 82% of the patients were males and 18% were females. The male to female ratio was 4.55:1

Table 2. Age distribution

Age group (Years)	Distribution (n=50)	
	Number	**Percentage**
30 or less	26	52.00
31 to 40	9	18.00
41 to 50	7	14.00
51 to 60	7	14.00
> 60	1	2.00
Total	**50**	**100.00**

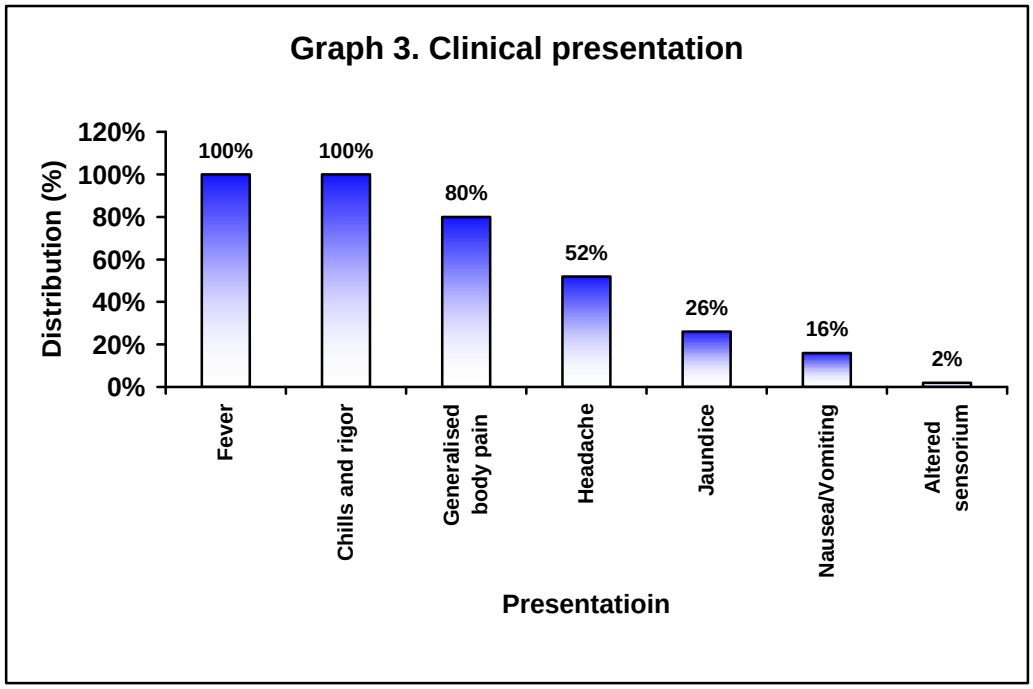


In this study the commonest age group at presentation was 30 years or less (52%). The next common age groups included 31 to 40 with 18% of the patients, 41 to 50 and 51 to 60 years comprised of 14% of the patients each. The mean age was found to be 33.96 ± 12.72 years.

Table 3. Clinical presentation

Presentation	Distribution (n=50)	
	Number	Percentage
Fever	50	100.00
Chills and rigor	50	100.00
Generalized body pain	40	80.00
Headache	26	52.00
Jaundice	13	26.00
Nausea / Vomiting	8	16.00
Altered sensorium	1	2.00

Multiple presentations hence total not shown



In the present study all the patients had fever with chills and rigors. Generalized body pain was present in 80%, headache in 52%, jaundice in 26% and nausea or vomiting in 16%.

Table 4. Clinical examination findings

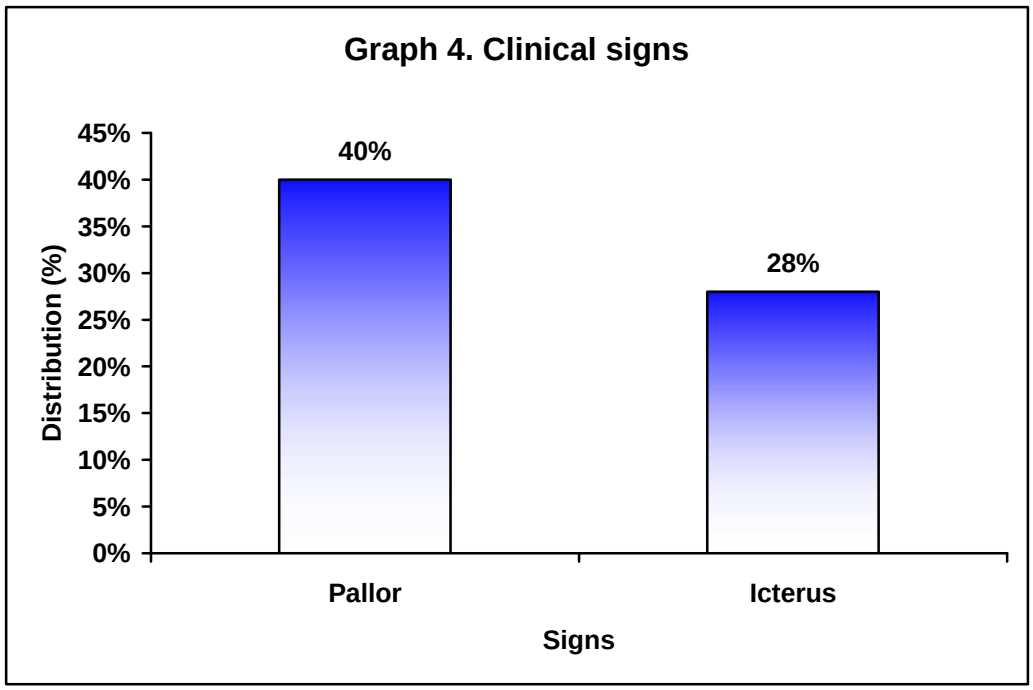
Vitals	Distribution (n=50)	
	Mean	**SD**
Heart rate (/Minute)	93.08	10.99
Systolic BP (mm Hg)	116.6	15.28
Diastolic BP (mm Hg)	72.72	8.04
Respiratory rate (/Minute)	20.88	3.34

The mean heart rate, systolic blood pressure, diastolic blood pressure and respiratory rate are as shown in table 4.

Table 5. Clinical signs

Signs	Distribution (n=50)	
	Number	**Percentage**
Pallor	20	40.00
Icterus	14	28.00

Multiple presentations hence total not shown

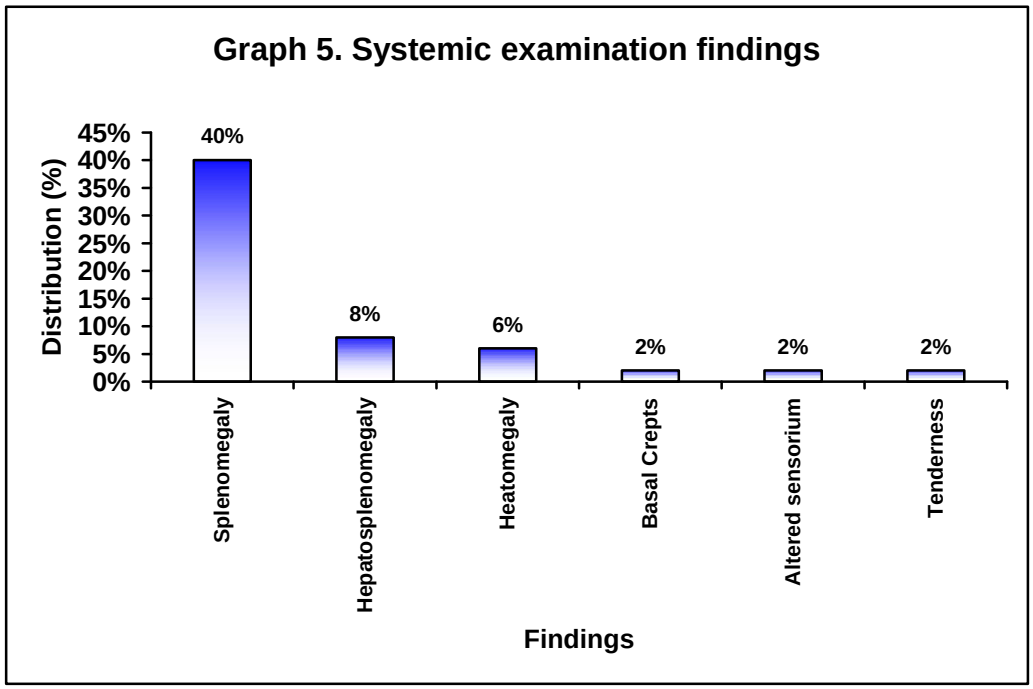


In this study the commonest clinical signs were noted as pallor (40%) and icterus (28%).

Table 6. Systemic examination findings

Findings	Distribution (n=50)	
	Number	Percentage
Only Splenomegaly	20	40.00
Hepatosplenomegaly	4	8.00
Only Hepatomegaly	3	6.00
Basal Crepts	1	2.00
Altered sensorium	1	2.00
Tenderness of abdomen	1	2.00

Multiple presentations hence total not shown

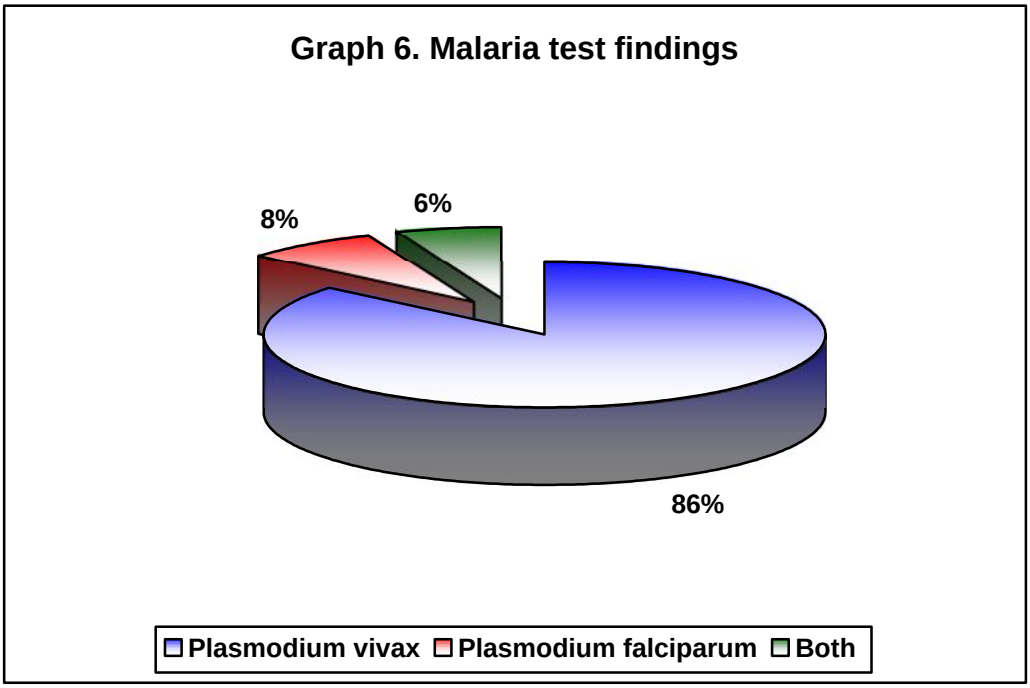


In this study, systemic examination findings revealed splenomegaly in 40%, hepatosplenomegaly in 8% and hepatomegaly in 6% of the patients. The

other findings noted were basal crepts, altered sensorium and tenderness of abdomen (2% each).

Table 7. Malaria test findings

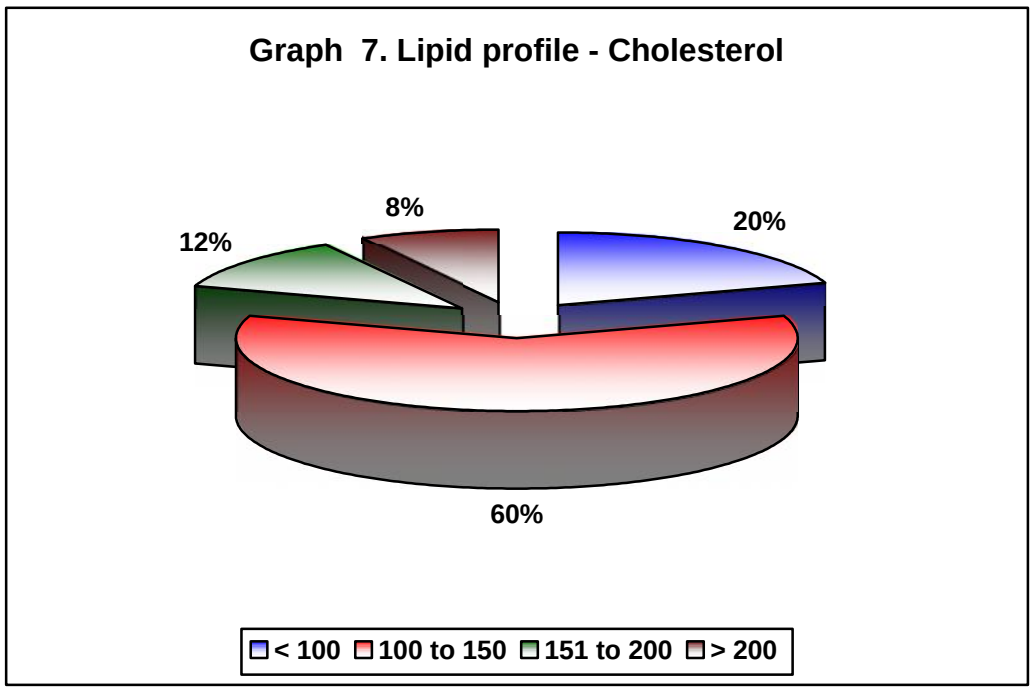
Findings	Distribution (n=50)	
	Number	**Percentage**
Plasmodium vivax	43	86.00
Plasmodium falciparum	4	8.00
Both (Plasmodium vivax and Plasmodium falciparum)	3	6.00
Total	**50**	**100.00**



In this study 86% of the patients were positive for plasmodium vivax and 8% for plasmodium falciparum. However, 6% of the patients were positive for both plasmodium vivax and plasmodium falciparum.

Table 8. Lipid profile - Cholesterol

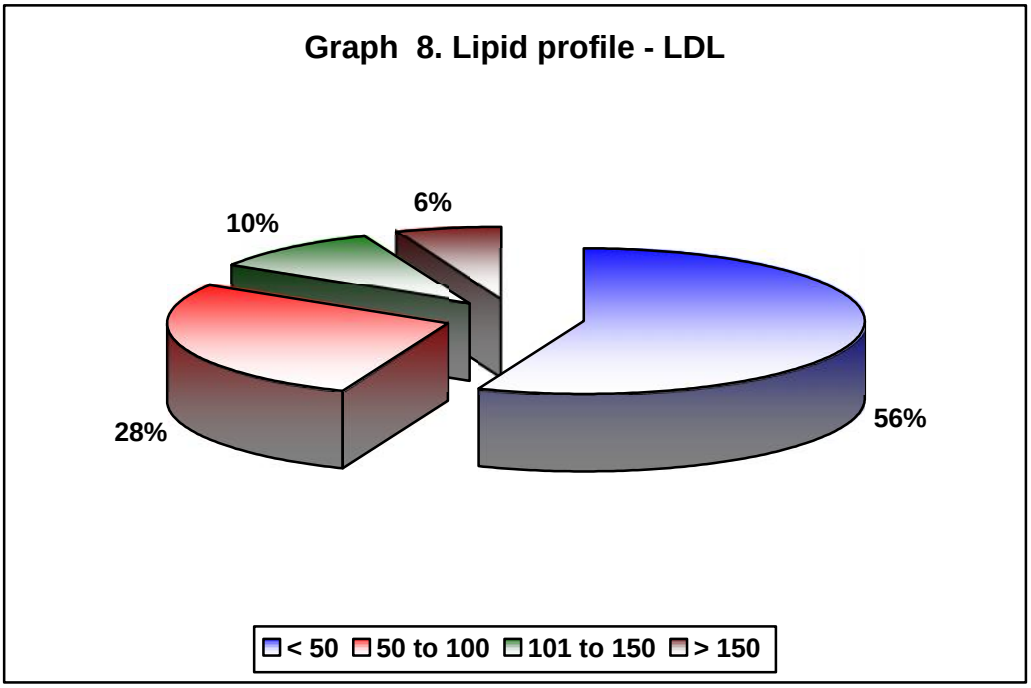
Total cholesterol levels (mg/dL)	Distribution (n=50)	
	Number	Percentage
< 100	10	20.00
100 to 150	30	60.00
151 to 200	6	12.00
> 200	4	8.00
Total	**50**	**100.00**



In the present study most of the patients (60%) had cholesterol levels between 100 to 150 mg/dL.

Table 9. Lipid profile - LDL

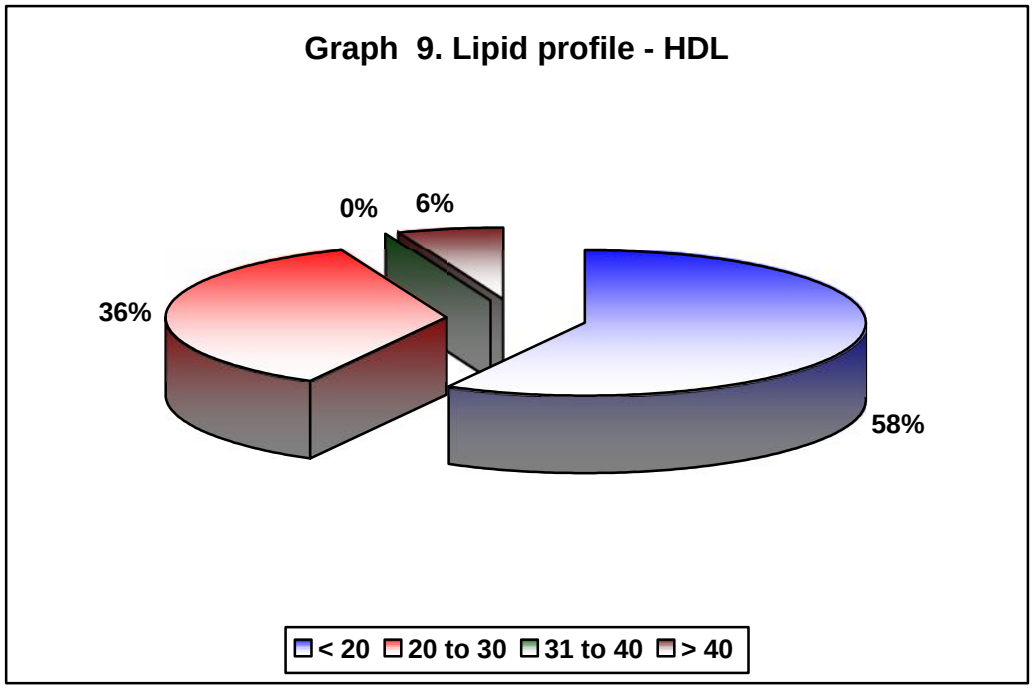
Low density lipoprotein levels (mg/dL)	Distribution (n=50)	
	Number	**Percentage**
< 50	28	56.00
50 to 100	14	28.00
101 to 150	5	10.00
> 150	3	6.00
Total	**50**	**100.00**



In this study 56% of the patients had LDL levels of less than 50 mg/dL.

Table 10. Lipid profile - HDL

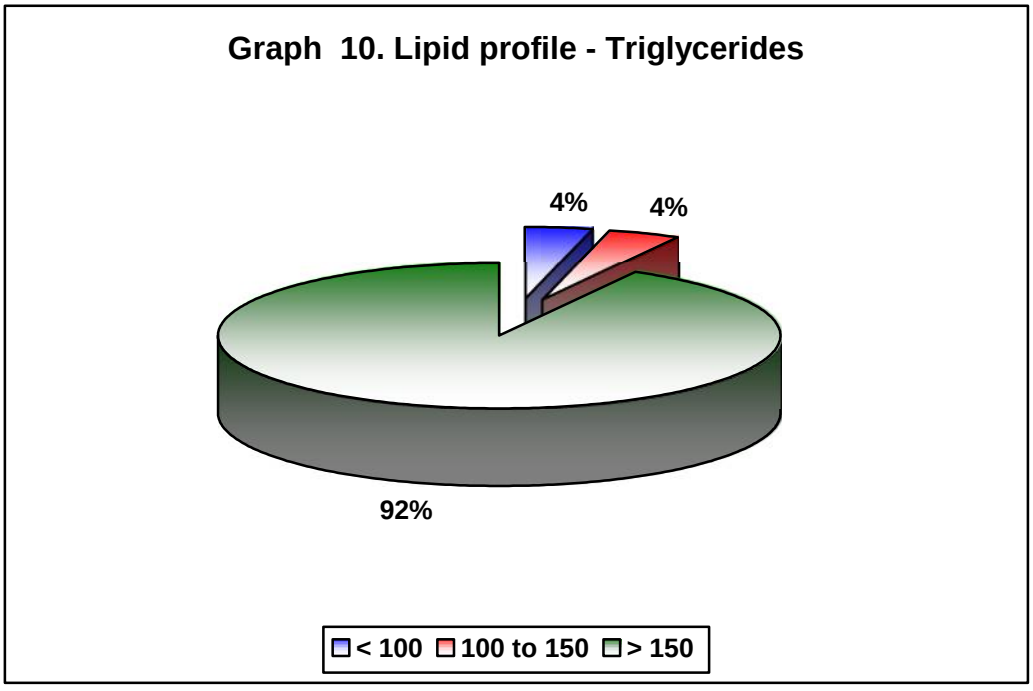
High density lipoprotein levels (mg/dL)	Distribution (n=50)	
	Number	Percentage
< 20	29	58.00
20 to 30	18	36.00
31 to 40	0	0.00
> 40	3	6.00
Total	**50**	**100.00**



In the present study HDL levels were less than 20 mg/dL in 58% of the patients while 36% of them HDL had levels between 20 and 30 mg/dL.

Table 11. Lipid profile - Triglycerides

Triglycerides (mg/dL)	Distribution (n=50)	
	Number	**Percentage**
< 100	2	4.00
100 to 150	2	4.00
> 150	46	92.00
Total	**50**	**100.00**



In this study majority of the patients (92%) had triglyceride levels more than 150 mg/dL.

Table 12. Characteristics of study population

Variables	Mean (n=50)		Mean (n=50)		
	Mean	**SD**	**Median**	**Min**	**Max**
Cholesterol (mg/dL)	126.00	41.07	113.00	65.00	246.00
LDL (mg/dL)	60.90	38.14	46.50	15.00	169.00
HDL (mg/dL)	19.66	12.08	17.50	7.00	79.00
Triglycerides (mg/dL)	274.38	141.57	228.00	53.00	710.00
Hemoglobin (gm %)	11.79	3.02	12.60	2.60	16.80
TLC (/Cumm)	7457.40	4244.43	6140.00	1900.00	22900.00
Platelet count (/Cumm)	92020.00	76958.91	63000.00	7000.00	263000.00
BUN (mg/dL)	31.38	15.27	27.50	12.00	89.00
Serum creatinine (mg/dL)	1.20	0.46	1.11	0.57	2.96
Total Bilirubin (mg/dL)	2.29	2.66	1.26	0.28	14.03
Direct Bilirubin (mg/dL)	1.18	1.91	0.43	0.03	10.30
SGOT (IU/L)	106.76	206.30	50.50	15.00	1303.00
SGPT (IU/L)	140.96	450.77	57.00	16.00	3204.00

The clinical characteristics of the study population are as shown in Table 12.

Table 13. Comparison of mean lipid parameters in sexes

Lipids	Males (n=41)		Females (n=9)		p value
	Mean	SD	Mean	SD	
Total cholesterol (mg/dL)	127.39	42.93	119.67	32.61	0.554
LDL (mg/dL)	63.46	39.19	49.22	32.33	0.270
HDL (mg/dL)	17.9	8.48	27.67	21.11	0.207
Triglycerides (mg/dL)	278.02	142.11	257.78	146.30	0.712

Table 13 shows comparison of mean lipid parameters in males and females. No statistically significant difference was observed in mean cholesterol, LDL, HDL and triglyceride levels ($p>0.050$).

Table 14. Comparison of mean lipid parameters in age groups

Age group (Years)	Lipids (mg/dL)							
	Total cholesterol		LDL		HDL		Triglycerides	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
< 30 (n=26)	105.46	17.87	43.12	18.43	20.50	10.29	240.00	119.80
31 to 40 (n=9)	123.33	33.09	64.22	44.60	14.33	3.50	348.11	141.97
41 to 50 (n=7)	159.14	59.64	84.14	52.62	16.86	4.38	306.29	137.75
> 50 (n=8)	166.75	42.36	94.63	35.99	25.38	22.97	275.25	193.26
F value	9.689		6.491		1.380		1.486	
p value	<0.001		<0.001		0.260		0.230	

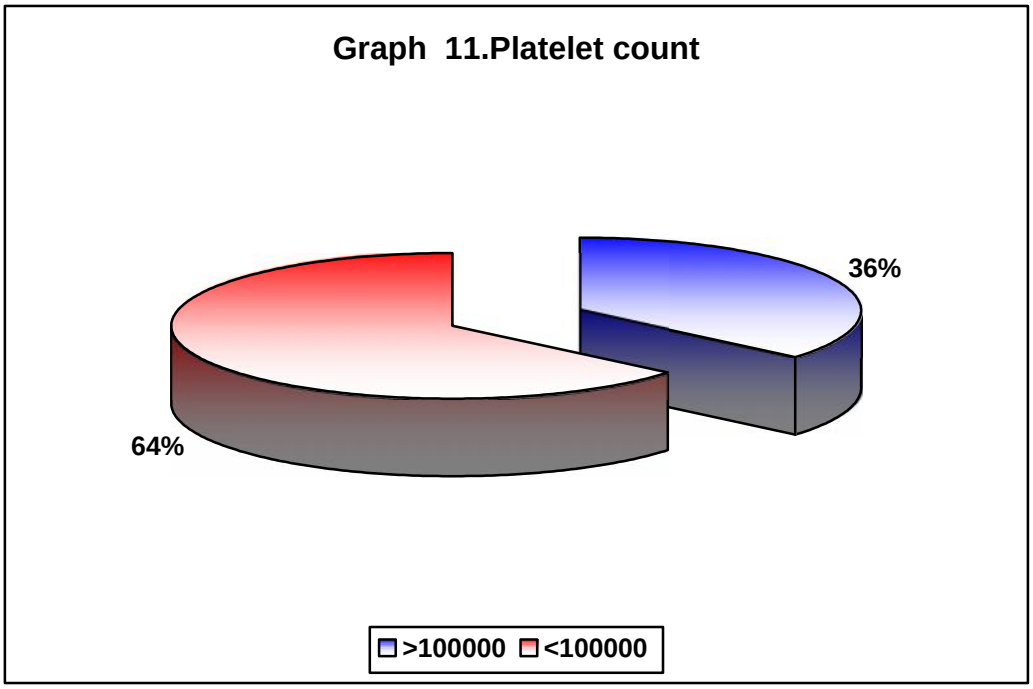
In this study the mean cholesterol levels in patients with age less than 30 years was 105.46 ± 17.87 mg/dL which increased to 166.75 ± 42.36 mg/dL in patients who were aged more than 50 years. This difference was statistically significant ($p<0.001$).

Similarly, the mean LDL levels in patients aged less than 30 years were 43.12 ± 18.43 mg/dL which increased to 94.63 ± 35.99 mg/dL in patients who were aged more than 50 years. This difference was statistically significant ($p<0.001$).

However, the mean HDL and triglyceride levels were comparable in all the age groups ($p>0.050$).

Table 15. Platelet count in Malaria infected patients

Platelet count (/cumm)	Distribution (n=50)	
	Number	**Percentage**
>100000	18	36.00
<100000	32	64.00
Total	**50**	**100.00**



In the present study 64% of the patients had platelet count of less than 100000 /cumm.

Table.16 Comparison of mean lipid parameters and platelet count

Lipids	Platelet count				p value
	<100000 (n=32)		>100000 (n=18)		
	Mean	SD	Mean	SD	
Total cholesterol (mg/dL)	116.03	24.38	143.72	57.08	0.064
LDL (mg/dL)	51.25	29.67	78.06	45.83	0.035
HDL (mg/dL)	19.19	9.67	20.50	15.77	0.751
Triglycerides (mg/dL)	293.16	157.65	241.00	102.98	0.165

Table 16 shows comparison of mean lipid parameters and platelet count. It was observed that, in patients with platelet count less than 100000 /cumm LDL levels were significantly low compared to patients with platelet count of more than 100000 /cumm (p=0.035). However, total cholesterol, HDL and triglycerides were comparable in both the subsets (p>0.050).

Chapter 6

Discussion

DISCUSSION

Malaria is recognized as a serious public health problem worldwide, affecting nearly 50% of the population in more than 109 countries. Studies have shown low levels of lipoproteins such as total cholesterol and high density lipoprotein (HDL), and low density lipoprotein (LDL) in patients suffering from malaria. The present study was aimed to assess the impact of malaria on lipid parameters.[127]

This one year cross sectional study was conducted on 50 adult patients presenting with clinical features suggestive of malaria infection in the Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum from January 2013 to December 2013.

In the present study majority of the patients were males 82% and 18% were females with male to female ratio of 4.55:1 suggesting male preponderance. Most of the point prevalence studies in India have been carried out for outbreak/epidemic investigations. There is very limited information on age and sex-specific seasonal prevalence of malaria in different paradigms in the country. In the available studies, age and sex classification used is arbitrary. The burden is generally higher in men than women in all age groups.[30]

More than half (52%) of the patients presented with age less than or equal to 30 years followed by 31 to 40 years (18%), 41 to 50 and 51 to 60 years (14% each). The mean age was found to be 33.96 ± 12.72 years. These findings were in agreement with a recent study[128] from North Maharashtra to assess the clinical profile of patients affected in P. falciparum, P. vivax and mixed infections. The

authors reported mean age as 36.76 ± 15.67 years with the predominant age group of 20-40 years comprising nearly 62% of the patients.

In this study all patients had fever with chills and rigors (100%). The next common clinical presentation was generalized body pain noted in 80% of the patients followed by headache in 52%, jaundice in 26% and nausea or vomiting in 16%. The commonest clinical signs noted were pallor (40%) and icterus (28%). The systemic examination revealed splenomegaly in most of the cases (40%) and few had hepatosplenomegaly (8%) and hepatomegaly (6%). However, basal crepitations, altered sensorium and tenderness of abdomen were present only in 2% of the patients each. In a recent study[128] from North Maharashtra, fever was the most predominant complaint i.e. 97% and 93% of the patients had chills and rigors. In the study conducted by Mehta et al[129] fever was present in 100% of the patients and it was present in 100% in the studies conducted by Malhotra et al.[130] Nausea and vomiting was observed in 16% of the patients with our study. It was 37.36% and 4.35% in study of Muddaiah et al[131] and Rathod et al[132] respectively. Muddaiah et al[131] and Rathod et al[132] reported 4.21% and 1.9% cases of altered sensorium in their studies respectively which were comparable with the present study.

Malaria is caused by protozoan parasites called Plasmodium, belonging to the parasitic phylum Apicomplexa. More than 200 species of the genus *Plasmodium* have been identified that are parasitic to reptiles, birds, and mammals.[132] Four Plasmodium species have been well known to cause human malaria, namely, *P.* falciparum, *P.* vivax, *P*. ovale, *and P*. malariae. A fifth one,

P. knowlesi, has been recently documented to cause human infections in many countries of Southeast Asia.[133]

In this study plasmodium vivax and plasmodium falciparum were positive among 86% and 8% of the patients respectively. However, 6% of the patients were positive on both the tests. Plasmodium falciparum is reported in 80-90% of cases in Africa, 40-50% of cases in western pacific and SE Asia, 4-30% in South Asia, South America and rest of tropics. In India, 30–90% of cases in Orissa, the NE states, Chattisgarh, Jharkhand, Madhya Pradesh, Bihar, and Andamans; <10% of cases have been reported from other areas. Plasmodium vivax is reported in 70-90% of cases in most of Asia and South America, 50-60% of cases in South East Asia and western pacific and 1-10% in Africa. In India, nearly 50% of total malaria burden; P. vivax is predominant species in most parts other than *P.* falciparum dominant areas.[134,135] However, in contrast to the findings of this study, a recent study from Karnataka to analyze and introspect the presentation of malaria reported P. vivax in 52.54%, followed by P. falciparum (33.75%) and mixed infections (13.69%).

In the present study nearly two third of the study population (60%) had cholesterol levels between 100 to 150 mg/dL. The mean and median cholesterol levels were noted as 126 ± 41.07 mg/dL and 113 mg/dL (Range 65 to 246 mg/dL) suggesting hypocholesterolemia. The mean cholesterol levels in patients aged less 30 years were 105.46 ± 17.87 mg/dL whereas those above 50 years had 166.75 ± 42.36 mg/dL suggesting as age advances the cholesterol levels increase ($p<0.001$). However the mean cholesterol levels were comparable in males and females ($p=0.554$). Previous studies done by Kittl et al,[136] Djoumessi[137] and

Nilsson et al[112] have shown the same relation between severe malaria and serum cholesterol. A review[15] reported that, serum total cholesterol which was measured in 36 studies and of them 83% (30/36 studies) reported hypocholesterolemia. Two studies reported a raised cholesterol,[93,119] three studies showed no significant differences with the control group[12, 121] and one study was inconclusive.[122] Authors commented that, cholesterol was significantly decreased during malaria. The findings of the present study were in agreement with these previous studies.

In this study, more than half of study population (56%) the LDL levels were found to be less than 50 mg/dL. The mean LDL levels were 60.90 ± 38.14 mg/dL and the median level was 46.50 mg/dL (15 to 169 mg/dL). No significant difference was noted in males and females (63.46 ± 39.19 and 49.22 ± 32.33 mg/dL respectively; p=0.270). But the mean LDL levels in patients aged less than 30 years was significantly low (43.12 ± 18.43 mg/dL) compared to those who were aged more 50 years (94.63 ± 35.99 mg/dL). Further, an increasing trend in LDL level was observed with advancing age ($p<0.001$). These findings suggest that, LDL level in patients with malaria is low and dependent on age of the patient. Recently, a review[15] including 16 studies assessed LDL in patients with malaria and 81% (13 out of 16 studies) reported a lower LDL concentration in malaria patients and concluded that LDL is significantly lower in malaria. A study performed by Krishna AP et al[93] found that there was a significant decrease in LDL cholesterol. The findings of the present study were in agreement with these studies.

In the present study, 96% of patients had HDL level below the normal range of 40mg/dl further in that, HDL level was less than 20 mg/dL (58%) in

most of the patients and almost one third of the study population had HDL levels between 20 to 30 mg/dL (36%). The mean (12.08 ± 12.08 mg/dL) and median HDL level (17.50 with range 7.00 to 79 mg/dL) were also suggestive of less than 20 mg/dL. These findings suggest that patients with malaria may have very low HDL level. However no statistically significant difference was observed in male and female as well as in different age groups ($p>0.050$). It is an established fact that acute infection and inflammation produce a moderate changes in plasma lipoprotein pattern in man, with a typical decline in HDL cholesterol. Faucher et al.[13] in 2002 reported that malaria infection produces moderate changes in plasma lipid profile in man, with typical decline in HDL concentration. It is worthwhile to note that Ogbodo et al.[138] (2008) proposed that oxidative modification of HDL and reduced serum levels of this class of lipoprotein was associated with the pathophysiology of malaria. Recently a review[15] of the 23 studies, measured HDL level in patients with malaria infection and 20out of 23 studies reported a significant decline in HDL level and concluded that HDL is significantly lower in malaria infected patients. A study performed by Krishna AP et al[93] also found that there was significant decrease in HDL cholesterol.

In this study, majority of the patients (92%) had triglyceride levels of more than 150 mg/dL. The mean and median triglyceride level were also suggestive of hypertriglyceridemia that is, mean triglycerides level was found to be 274.38 ± 141.57 mg/dL and median level was 228 mg/dL ranging between 53 and 710 mg/dL. However, these observations in different sexes and age groups revealed no statistically significant difference ($p>0.050$). Studies done by Parola et al[139] and Onongbu et al[120] have shown similar results. A study performed by

Krishna AP et al[93] found that there was significant raise in triglycerides. A review of 23 case-control studies, observed that there was a significantly higher mean triglyceride plasma concentration in malaria patients compared to controls and concluded that triglycerides are significantly higher in malaria patients compared to healthy controls.

Interestingly, in the present study 64% of the patients had platelet count of less than 100000 /cumm suggesting thrombocytopenia. Among them the mean LDL level was significantly low compared to patients having platelet count of more than 100000 /cumm (51.25 ± 29.67 vs. 78.06 ± 45.83 /cumm; p=0.035) but, total cholesterol, HDL and triglycerides were comparable in both the subsets.

Overall the present study showed that patients with malaria are prone to lipid derangement with hypocholesterolemia, lower LDL and HDL cholesterol and hypertriglyceridemia. Several other studies have reported similar observations.

A study performed by Krishna AP, et al.[93] to assess the lipid parameters in malaria infected patients found that there is significant decrease in HDL cholesterol and LDL cholesterol and increase in triglycerides.

A study performed by Sibmoooh N, et al.[124] reported alteration of lipid metabolism during acute malarial infection which may be the result of acute phase response. Further the acute phase response is associated with changes in lipid metabolism including a moderate increase in serum triglycerides and VLDL, but decrease in HDL and LDL.

Another study done by Sin C et al[125] noted significant decrease in serum HDL in malaria infected patients and concluded that serum HDL may be valuable information in the diagnosis of the malaria.

Mohanty S et al.[111] conducted a case control study, to assess malaria induced changes in plasma lipids and found that there was decrease in LDL and HDL in cases when compared to controls.

However, the mechanism involved in lipid changes related to malaria remains still uncertain. It has been concluded that they may be partly host related, i.e. related to an acute phase reaction or may be selective uptake of HDL particles by the Plasmodium.[93]

The present study extends the observation of earlier studies and clearly demonstrates that acute malarial infection produces a unique perturbation of plasma lipoprotein metabolism and reflects a multiple alterations in lipid and lipoprotein profiles in plasma. This further justifies the hypothesis looking for plasma lipid profiles in the diagnosis of malarial infection.

Chapter 7

Conclusion

CONCLUSION

Malaria continues to be a huge social, economical and health problem, particularly in the tropical countries. The present techniques to diagnose malaria infection vary in sensitivity and specificity and these techniques have their own advantages and disadvantages with respect to time consumption, cost effectiveness and ease of procedure. The patients with malaria infection are prone to have significant hypertriglyceridemia, hypocholesterolemia, and low HDL and LDL levels. Therefore significant variation in common lipid profile in malaria patients may serve as tool for diagnosis of malaria infection.

In our study, assessment of lipid profile in 50 adult malaria patients showed promising results with hypocholeterolemia in 60% of the study population, low LDL (less than 50mg/dl) in 56% of patients and low HDL (less than 40mg/dl) in 96% of patients. Whereas 92% of patients had hypertriglyceridemia. Interestingly in this study, it was found that thrombocytopenia was more significant (p value 0.035) in malaria patients with low LDL levels. Although mechanism underlying these changes is not completely understood and remains unclear. Increased research studies to elucidate the accurate mechanism responsible for these lipid alterations are warranted.

Hence it may be concluded that, serum lipid profile changes are a characteristic feature of malaria infection and these findings need to be further analyzed through case-control studies which could lead to enhanced understanding of the disease and consequently novel diagnostic and treatment methods.

Chapter 8

Summary

SUMMARY

The impact of malaria on variation of serum lipid profile parameters in malaria infected patients has been proposed in previous studies. Though the underlying biological mechanism remains unclear. The present study was aimed to assess the lipid profile in patients with malaria infection.

The present one year cross sectional study was undertaken on a total of 50 adult patients presenting with clinical features suggestive of malaria infection from January 2013 to December 2013 in the Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum. All these patients were investigated for serum lipid profile.

In this study young males were predominantly affected (82%), and the male to the female ratio was 4.55:1, the mean age was 33.96 ± 12.72 years. All the patients at presentation had fever with chills and rigors (100%) and the next common presentation was a generalized body pain (80%). The commonest clinical sign was pallor (40%).

On systemic examination, splenomegaly was noted in 40% of patients. The majority of the patients (86%) were positive for Plasmodium vivax.

In our study, 60% of malaria infected patients were found to have lower total cholesterol levels, the values being between 100 to 150 mg/dL, where 56% of the patients had hypolipidemia with LDL levels of less than 50 mg/dL. Furthermore, 96% of patients had HDL level below the normal range of 40mg/dl further in that, 58% of patients had very low HDL of value less than 20mg/dl

whereas 92% of the patients had hypertriglyceridemia with triglyceride levels of more than 150 mg/dL.

Platelet count was < 100000 in 64% of the patients. Interestingly patients with thrombocytopenia had lower LDL cholesterol.

In patients with thrombocytopenia, the mean LDL levels were significantly lower compared to patients with platelet count of > 100000 /cumm (51.25 ± 29.67 vs. 78.06 ± 45.83 /cumm; p=0. 035).

Overall, in patients with malaria infections, variation in lipid parameters with hypocholesterolemia, low HDL, low LDL levels and hypertriglyceridemia were evident.

Chapter 9

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Annexures

Annexure II

ANNEXURE II – PROFORMA

1. Sl. No

2. Name:

3. Age:

4. Sex

5. Occupation:

6. Religion:

7. In patient/Out patient Number:

8. Address:

9. Date of admission: 10. Date of discharge:

History:

Fever	yes/no
Chills and rigors	yes/no
Jaundice	yes/no
Generalized body pains	yes/no
Headache	yes/no
Nausea / vomiting	yes/no
Altered sensorium	yes/no
Decrease urine output	yes/no
Any other symptom:	

Past history:

Treatment history:

Lipid lowering drugs: Yes / no

Personal history:

H/o smoking:	Yes / no
H/o alcohol consumption	Yes/ no

General condition:

Pallor	Yes/no
Icterus	Yes/no
Cyanosis	Yes/no

Vitals:

Temperature:

Pulse:

Respiratory rate:

Blood pressure:

Systemic examination:

CVS:

Respiratory system:

Per abdomen:

CNS:

Investigations:

Malaria Card Test or PS For M.P	
LIPID PROFILE	
TOTAL CHOLESTEROL	
LDL	
HDL	
TRIGLYCERIDES	
HAEMOGLOBIN	
RED CELL COUNT	
TOTOL LEUCOCYTES	
NEUTROPHILS	
LYMPHOCYTES	
EOSINOPHILS	
MONOCYTES	
BASOPHILS	
PLATELETS	
UREA	
SERUM CREATININE	
SODIUM	
POTASSIUM	
TOTAL BILIRUBIN	
DIRECT BILIRUBIN	
S.G.O.T	
S.G.P.T	

Annexures

Annexure III

ANNEXURE III – MASTER CHART

-	-	Absent
+	-	Present
BC	-	Bilateral crepts
BP	-	Blood pressure
Cumm	-	Cubic millimeter
dL	-	Deciliter
F	-	Female
gm	-	Gram
HPL	-	Hepatomegaly
IU/L	-	International units per litre
M	-	Male
mg	-	Milligram
mm Hg	-	Millimeters of mercury
N	-	Normal
N	-	Normal
SGOT	-	Serum glutamic oxaloacetic transaminase
SGPT	-	Serum glutamic pyruvic transaminase
SPL	-	Splenomegaly
TEND	-	Tenderness

Annexures

Annexure I

ANNEXURE I – CONSENT FORM

"VARIATION IN COMMON LIPID PARAMETERS IN MALARIA INFECTED PATIENTS A ONE YEAR CROSS SECTIONAL STUDY."

Objective and purpose of the study:

This research is intended to estimate the lipid profile in patients with malaria infection. The principal investigator of the study is conducted by Post Graduate student in M. D. in GENERAL MEDICINE,

Procedure:

If you agree to be part of the research study you will be asked the relevant history and will be subjected to relevant clinical examination and investigations. You will also have to give blood sample.

Risk and Benefits:

The only risk and possible discomfort you might get is while taking blood from your arm for the investigations. It may cause swelling, pain, redness, bruising or infection (rarely happens) at the site from where the blood is drawn.

Alternatives

Taking part in this study is voluntary. You may choose not to take part in this study, or if you decide to take part you can later change my mind and withdraw from the study. Your decision will not change the present or future health care or other services that you receive. The study doctor or sponsorer may stop your participation in this study any time. If you choose not to take part in the study you will receive the standard treatment for patients with your condition.

VOLUNTARY PARTICIPATION/ WITHDRAWAL:

Your participation in this study is entirely voluntary and you may withdraw from the study at any time.

Privacy and Confidentiality

All information collected about you during the course of this study will be kept confidential to the extent permitted by law. The code numbers will identify you in this research record. Information from this study may be published but your identity will be confidential in any publication.

Institution / Sponsor's policy

Does not apply to this research

Financial incentives for participation

You will not be paid / offered any gifts /incentives for participating in the study.

Authorization to publish the results

The results of the study would be forwarded to the KLE University, Belgaum as part of requirement towards the completion of MD degree, review and publishing.

Questions

If you have any questions about the research you may please contact:

1. Investigator, _____ Post Graduate student, Department of Ophthalmology, JNMC, Belgaum.
2. Guide, _____ Professor, Department of Ophthalmology, JNMC, Belgaum
3. _____ Principal, JNMC, Belgaum and Chairman, Institutional Ethics Committee.

CONSENT FORM

I voluntarily agree to take part in this study by signing on the line below. I may withdraw at any time. I am not giving up any of my legal rights by signing this form. My signature below indicated that I have read this entire consent form or it has been read to me, and has been explained to me in my vernacular language and had all my questions answered. I will be given a copy of this consent form.

Signature /Left Thumb print of the Participant or legally authorized representative.

Participant's Name/ : …….

Signature/ Left Thumb

Impression of the participant's : …….

Name of the legally

Authorized representative/ Guardian : …….

Signature/ Left Thumb Impression. : …….

Witness's Name : …….

Signature/ Left Thumb Impression. :…….

Investigators name and Signature : …….

Date and Place : …….