STUDY OF THROMBOCYTOPENIA IN MALARIA- CORRELATION WITH TYPE AND SEVERITY OF MALARIA AND ITS PROGNOSTIC SIGNIFICANCE

A dissertation submitted to the

BLDE UNIVERSITY, BIJAPUR

In partial fulfillment of the requirements for the award of

M. D. DEGREE (Internal Medicine),

Examination to be held in

April 2012.

Submitted by

Dr. Nijora Deka

Under the guidance of

Dr. R. C. Bidri

B. L. D. E. U'S

SHRI B. M. PATIL MEDICAL COLLEGE HOSPITAL & RESEARCH CENTRE, BIJAPUR.

B. L. D. E. U'S

SHRI B. M. PATIL MEDICAL COLLEGE HOSPITAL & RESEARCH CENTRE, BIJAPUR.

DEPARTMENT OF MEDICINE

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation/thesis entitled **"STUDY OF THROMBOCYTOPENIA IN MALARIA - CORRELATION WITH TYPE AND SEVERITY OF MALARIA AND ITS PROGNOSTIC SIGNIFICANCE"** is a bonafide and genuine research work carried out by me under the guidance of **DR. R.C. BIDRI,** Principal and Professor in the Department of General Medicine at Shri B. M. Patil Medical College Hospital &Research Centre, Bijapur.

Date:

Place: BIJAPUR

DR. NIJORA DEKA

B. L. D. E. U'S

SHRI B. M. PATIL MEDICAL COLLEGE HOSPITAL & RESEARCH CENTRE, BIJAPUR.

DEPARTMENT OF MEDICINE

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This is to certify that the dissertation entitled "**STUDY OF THROMBOCYTOPENIA IN MALARIA - CORRELATION WITH TYPE AND SEVERITY OF MALARIA AND ITS PROGNOSTIC SIGNIFICANCE** " is a bonafide research work done by **DR. NIJORA DEKA** in partial fulfillment of the requirement for the degree of M. D. Internal Medicine to be held on April 2012

Date:

Place: BIJAPUR

Dr. R. C. BIDRI MD

PRINCIPAL & PROFESSOR,

DEPARTMENT OF MEDICINE

BLDEU'S SHRI B.M. PATIL MEDICAL COLLEGE HOSPITAL AND RESEARCH CENTRE, BIJAPUR

B. L. D. E. U'S

SHRI B. M. PATIL MEDICAL COLLEGE HOSPITAL & RESEARCH CENTRE, BIJAPUR.

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DR. M.S. BIRADAR

HEAD OF DEPARTMENT,
B. L. D. E. U'S SHRI B. M. PATIL MEDICAL COLLEGE HOSPITAL & RESEARCH CENTRE, BIJAPUR.

Date:

Place: BIJAPUR

DR. R.C. BIDRI

PRINCIPAL,
B. L. D. E. U'S SHRI B. M. PATIL MEDICAL COLLEGE HOSPITAL & RESEARCH CENTRE, BIJAPUR.

Date:

Place: BIJAPUR

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Place: BIJAPUR

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Place : BIJAPUR

Date: **Dr. NIJORA DEKA**

LIST OF ABBREVIATIONS USED

(in alphabetical order)

DIC-Disseminated intravascular Coagulation

Hb – Hemoglobin

P.F- Plasmodium Falciparum

PfEMP-1 P. f Erythrocyte Membrane Protein 1

Pf HRP-Plasmodium Falciparum histidine rich protein

pLDH-Plasmodium Lactate Dehydrogenase

PMA-Pan Malarial Antigen

P.V-Plasmodium vivax

TRAP-Thrombospondin-Related Adhesive Protein

WHO-World Health Organization

ABSTRACT

BACKGROUND

Malaria is one of the most important of the tropical diseases, remaining widespread throughout the tropics. It is a major public health problem in the world and continues to afflict the poor nations and the poor most. Malaria is a curable disease if the patients have access to early diagnosis and prompt treatment.[6]It has been highlighted in various studies that thrombocytopenia which is commonly seen in malaria is a reliable diagnostic marker. However the prognostic implications could vary in the two types of malaria. Statistically, low platelet levels are seen more commonly in P. falciparum malaria and this can have therapeutic implications.

We attempt to evaluate the occurrence and severity of thrombocytopenia in hospitalized patients of malaria and correlate association of low platelet counts with the type of malaria and its prognostic implications.

OBJECTIVES:

1. To study the incidence of thrombocytopenia in malaria

2. To correlate severity of thrombocytopenia with type of malaria and its prognostic significance.

METHODS:

A total of 70 patients diagnosed to have Malaria over a period of two years admitted in Shri B.M. Patil Medical College & Hospital were studied. All study subjects were identified positive for malaria parasite on peripheral smear examination with conventional microscopy.Platelet count was done on a fully automated, quantitative analyzer. Daily platelet count was done for all those admitted with malaria. P.falciparum antigen test (PfHrp antigen test-Parascreen) was performed in subjects with P.vivax Malaria on the peripheral smear with a platelet count less than 20,000cells/cmm for more emphatic exclusion of associated P.falciparum infestation. P.falciparum antigen test was also performed in subjects with high index of clinical suspicion or multi organ involvement

Results:

In the study, a total of 70 patients were found to have malaria, 38(54.2%) were P.vivax, 24 (34.3%) were P.falciparum and 2(2.8%) were mixed. 91.4% patients had thrombocytopenia. 16(25%) developed complicated malaria. Severe thrombocytopenia was noted in 58.8% of complicated malaria with $p<0.003$. 12 patients persisted to have thrombocytopenia on 6th day even after adequate therapy. 8(80%) patients recovered and 1(10%) died which was P.falciparum.

Interpretation and conclusion:

Thrombocytopenia is a common association of malaria with incidence of 91.4%. Severe thrombocytopenia is commonly seen in P.falciparum. Platelet count <25,000 was not seen in P.vivax. Out of 13 severe thrombocytopenia 10 developed complicated malaria with significant p value indicating that patients with severe thrombocytopenia at the time of admission are more prone to develop complications when compared to mild and moderate thrombocytopenia. Patients who persisted to have thrombocytopenia even after 6th day of therapy, their mortality increased by 10%.

KEY WORDS: Malaria; Thrombocytopenia.

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1. Introduction

Malaria has been one of the most prominent and ancient diseases which has been profiled and studied. It is an infectious disease caused by a parasite, *Plasmodium*, which infects red blood cells and is characterized by cycles of fever, chills, pain, and sweating.

William Osler said, **"Humanity has but 3 great enemies; fever, famine and war; of these by far the greatest, by far the most terrible is fever"** [10]

Malaria, unlike many diseases, has evolved as a result of millions of years of interactions at various levels with the mammalian system and hence has found ways to gather resistance to drugs and insensitivity to other treatment modalities. Co-evolution and co-adaptation of the parasite, recrudescence, recurrence, drug resistance, and the complex pathophysiology of the infection process complicate treatment regimens.

Malaria plagued the mankind in the past. Now though ignored as a simple disease, one should not forget that it still has vampirical outcomes in many cases. Malaria is still an endemic disease, especially in the African tropics and India. It is also endemic in many parts of Karnataka.

- Malaria causes significant economic losses, and can decrease gross domestic product (GDP) by as much as 1.3% in countries with high levels of transmission [5]
- In some heavy-burden countries, the disease accounts for:
    - up to 40% of public health expenditures;
    - 30% to 50% of inpatient hospital admissions;
    - up to 60% of outpatient health clinic visits. 5

- 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.
- International funding for malaria control has risen steeply in the past decade. Disbursements reached their highest ever levels in 2009 at US$ 1.5 billion to US$ 1.8 billion in 2010. The

amounts committed to malaria, while substantial, still fall short of the resources required for malaria control, estimated at more than US$ 6 billion for the year 2010.3

A prompt and early diagnosis is key to effective management in malaria. Many acute febrile illnesses like viral fever, arboviral infections, enteric fever and leptospirosis occur in the tropics and it is difficult to distinguish malaria from these illnesses on clinical grounds alone. Microscopic diagnosis by peripheral smear examination is used for diagnosis. However it needs expertise and needs repeated examination to rule out malaria. It's a valuable technique when performed correctly but wasteful when poorly executed and when in less expert hands.

It has been highlighted in various studies that thrombocytopenia which is commonly seen in malaria is a reliable diagnostic marker. However the prognostic implications could vary in the two types of malaria. Statistically, low platelet levels are seen more commonly in P. falciparum malaria and this can have therapeutic implications.

Over the last decade, a number of studies show that platelets play a role in the pathophysiology of severe malaria. Radioactive-labeled platelet studies indicate enhanced production of thromboxane A2 and prostacyclin (PGI2) by platelets leading to a hypercoagulable state in malaria.

Studies performed show that platelets in cerebral microvessels adhere to the surface of TNF-stimulated endothelium to form bridges between P. falciparum infected erythrocytes (PfIE) and cerebral endothelium by providing new parasite receptors to brain endothelial cells initially devoid of them. Advances in receptor blockade for anti malarial therapy bring in the possibility of development of new therapies and prognostic tools.

There have been great advances in medical field and we have also been able to understand malaria better. There has been a lot of development in antimalarial drugs. Even with these advances we are not able to completely control or eradicate malaria. Still many deaths occur due to malaria. In the last few decades an effort has been made to produce an effective malaria vaccine. These are still at developmental stages.

2. AIMS AND OBJECTIVES:

1. To study the incidence of thrombocytopenia in malaria

2. To correlate severity of thrombocytopenia with type of malaria and its prognostic significance.

3. HISTORICAL REVIEW

Malaria is a major public health problem in India. Intermittent fever, with high incidence during the rainy season, coinciding with agriculture, sowing and harvesting, was first recognized by in the 18th century Romans and Greeks who associated it with swampy areas. They postulated that intermittent fevers were due to the 'bad odour' coming from the marshy areas and thus gave the name 'malaria' (Mal – Bad, Aria – Air) to intermittent fevers. [10] In spite of the fact that today the causative organism is known, the name has stuck to this disease. [10]

Hippocrates (470-377 B.C) is credited with the first clear description of malaria. In his 'Aphorisms' he described the regular paroxysms of intermittent fever.

Charaka and Shushruta (1500-800 B.C) renowned Indian physicians gave vivid descriptions of the disease many centuries ago.

The treatment of the disease became first established (in the middle of the seventeenth century) before anything was known about its etiology and how the disease was transmitted.

Another curious fact is that before the discovery of the plasmodia, the presence of pigment (black material) in organs with malarial infections had been observed by Meckel (1847) and Virchow (1849). The pigmented appearance of spleen and brain in malaria post mortem was however noted by Lancisi in 1716 and Bright in 1831.

The following are the landmarks in the history of malaria.7

1880 - Laveran discovered the malaria parasite in an unstained preparation of fresh blood.

1883 - Marchiafava used methylene blue for staining the malaria parasite.

1885 - Golgi demonstrated erythrocytic schizogony (Golgi cycle) of quartan malarial parasite.

1886 - Golgi described the erythrocytic schizogony of benign tertian malaria parasite, also differentiated the benign and quartan species.

1891 - Romanowsky introduced the staining method of malaria parasite

1897 - Ross in Secunderabad found oocysts in the stomach wall of female anopheline mosquito which had previously fed on a malaria patient.

1898 - Ross in Calcutta worked out the mosquito cycle of the parasite of bird malaria, whereas Bignami et al demonstrated the same with the parasite of human malaria.

1900 - Patrick Manson proved the theory of mosquito transmission.

1934 - Tissue phase of malaria parasite was demonstrated in avian malaria

948 - Shortt and others worked out pre-erythrocytic schizogony of malaria parasite in the parenchymal cells of liver, first with cynomolgi malaria then with vivax malaria. They also demonstrated the exo-erythrocytic schizogony of malaria parasite in cynomolgi malaria.

1949 - Shortt and others demonstrated the pre-erythrocytic schizogony of P. falciparum.

1952 – Jeffery et al demonstrated a 3 day old pre-erythrocytic schizont of P. falciparum in human liver.

1954 - Garnham et al discovered the pre-erythrocytic schizogony of P. ovale.

Treatment

Cinchona bark was used to treat fever in 17th century, later was recognized to be specific for malaria.

Quinine was isolated from cinchona bark in 1820. It was a major anti malarial till 1942.

Mepacrine was produced in Germany in 1926.

Primaquine was produced in Germany in 1920's.

Chloroquine was produced and used by Germans from 1934.

Pyrimethamine was produced in 1951.

Recently mefloquine, artemesinin derivatives were introduced.

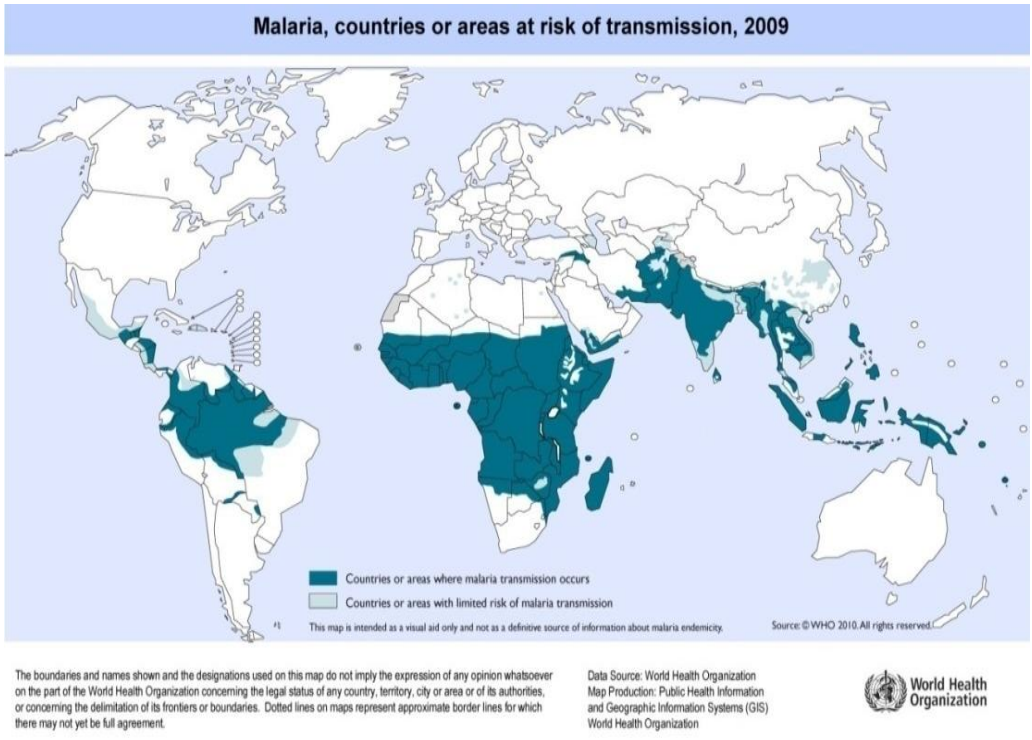
EPIDEMIOLOGY

"Everything about malaria is so moulded by local conditions that it becomes a thousand epidemiological puzzles." Hackett (1937)

The above quote emphasizes the complexity of malaria and the many facets the disease exhibits. Different communities will experience different malaria and consequently different control and treatment strategies may be necessary. The intricate interactions between host, parasite, and vector are the major factors in this epidemiological complexity.

Present in over 100 countries, mainly tropical. It is found in all countries extending from 40 degree South to 60 degree North. Tropical zone is endemic for all forms of malaria. Plasmodium malariae is endemic in Sub tropical zone. Plasmodium vivax is endemic in temperate zone. Plasmodium ovale is endemic in East Africa, West Africa, Philippines.

Fig. 1: Epidemiology: Malaria map WHO



Malaria transmission does not occur at

- Temperature below 16 degreeC or above 33 degree C. Sporogony does not take place in the mosquitoes under these conditions. Optimum conditions for transmission are high humidity and an ambient temperature between 20 degree C and 30 degree C.
- Altitude greater than 2000 m.

Sl. No.	Endemicity	Spleen rate	Parasite rate
1.	Hypoendemic	0-10%	0-10% in children aged 2-9 years.
2.	Mesoendemic	11-50%	11-50% in children aged 2-9 years.
3.	Hyperendemic	51-75%	51-75% in children aged 2-9 years, adult spleen rate is high
4.	Holoendemic	Over 75%	Over 75% in children, adult spleen rate low. Parasite rate in 1st year of life is high

Table 1: Classification of Endemicity 10

The endemicity of malaria is defined traditionally in terms of spleen or parasite rate in children between 2 and 9 years.

Stable or endemic malaria

- sustained incidence over several years
- includes seasonal transmission
- various levels of endemicity
- immunity and disease tolerance correlate with endemicity , epidemics unlikely

Unstable or epidemic malaria

- marked increase in incidence
- population is non-immune
- morbidity and mortality can be high

- About 3.3 billion people i.e. nearly half of the world's population are at risk of malaria.4

- In 2008, there were 247 million cases of malaria and nearly one million deaths.5 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. The tropics provide ideal breeding and living conditions for the anopheles mosquito, and hence this distribution.[1]
- The WHO forecasts a 16% growth in malaria cases annually [1,2]
- About 1 million people die of malaria every year (85% of these occur in Africa), accounting for about 4-5% of all fatalities in the world. In Africa a child dies every 45 seconds of Malaria, the disease accounts for 20% of all childhood deaths.[1]
- Malaria kills in 1 year what AIDS killed in 15 years. In 15 years, if 5 million have died of AIDS, 50 million have died of malaria [2,3]
- There is one malarial death every 12 sec somewhere in the world.
- Malaria ranks third among the major infectious diseases in causing deaths- after pneumococcal acute respiratory infections and tuberculosis.
- South-East Asia Region has a malaria burden second only to Africa 2 . During 2009 estimated malaria cases were around 26 -36 million and malaria deaths between 42300 – 77300. 6
- Incidence in India: There were 6.74 million cases in 1976. It decreased to 2.1 million in1984. There has been no further significant decrease in the annual incidence [8].
- India accounts for 73% of the reported cases and 28% of the total reported deaths in the in the South-East Asia Region [2]. Approximately 95% of the country's population lives in malaria-endemic areas; 80% of malaria occurs among 20% of the population who are classified as 'high risk populations residing in Andhra Pradesh, Chhatisgarh, Gujarat, Jharkhand, Madhya Pradesh, Maharashtra, Orissa, Rajasthan, the seven north-eastern states and Sikkim [2]
- Pregnant women are at high risk not only of dying from the complications of severe malaria, but also spontaneous abortion, premature delivery or stillbirth. Malaria is also a cause of severe maternal anaemia and is responsible for about one third of preventable low birth weight babies.

Table2: Incidence of malaria, deaths in last 15 years in India [9].

Year	Total Malaria Cases (million)	P.falciparum cases (million)	Deaths due to malaria
1995	2.93	1.14	1151
1996	3.04	1.18	1010
1997	2.66	1.01	879
1998	2.22	1.03	664
1999	2.28	1.14	1048
2000	2.03	1.05	932
2001	2.09	1.01	1005
2002	1.84	0.90	973
2003	1.87	0.86	1006
2004	1.92	0.89	949
2005	1.82	0.81	963
2006	1.79	0.84	1707
2007	1.51	0.74	1311
2008	1.53	0.77	1055
2009	1.56	0.84	1144
2010 (till Nov)	1.37	0.69	678

*Data for 2009 & 2010 are Provisional

Trend of Malaria Cases And Deaths 2001-2009 in India[9]

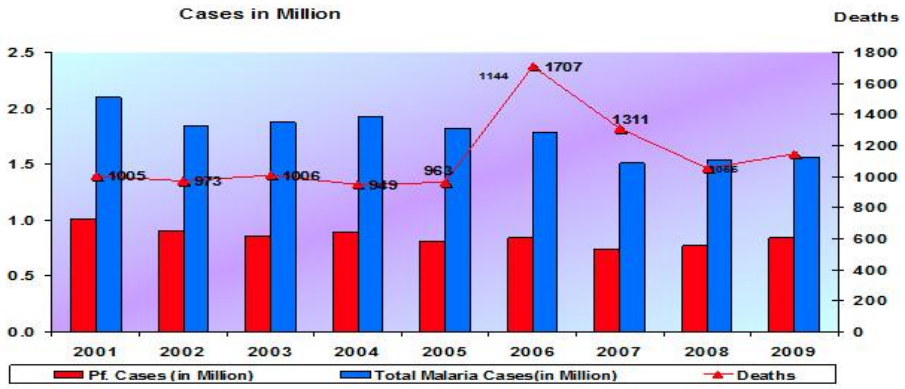


Table 3; Incidence of Malaria in Karnataka in from 2006- 2010 [9].

*Data for 2009 & 2010 are Provisional

Malaria problem in Karnataka :

Malaria is one of the major communicable diseases in Karnataka. The disease was at its peak in 1976 with about 6,30,000 cases reported in the state. It showed study decline till 1984 when only 30,000 cases were reported. During the year 1997, the total reported case incidence was 1,80,000 for the state. During the year 1998, 1,10,000 cases of malaria were reported in the state of which 25,000 were due to P. falciparum.

The districts Kolar, Bijapur, Mandya, Bellary and Raichur contributed 63% of the total malaria cases in 1997 and 75% of the P. falciparum cases. Unlike in some other states malaria is predominantly a rural problem in our state.

Year	**Total cases**	***Pl.falciparum cases***
2006	62842	16459
2007	49355	11295
2008	47344	9864
2009	36859	5723
2010 (upto Nov)	41861	7268

REVIEW OF LITERATURE

Anopheles Mosquito:

Malaria is transmitted from man to man by the female anopheles mosquito, one of themost capable vectors of human disease. Nearly 45 species of the mosquito have been found in India and A. culicifacies, A. fluviatilis, A. minimus, A. philippinensis, A. stephensi, A.sundaicus, and A. leucosphyrushave been implicated in the transmission of malaria.

The areas of distribution are different for these mosquitoes:

A. fluviatilis, A. minimus arefound in the foot-hill regions,

A. stephensi, A. sundaicus are found in the coastal regions,

A. culicifacies and A. philippinensis are found in the plains.

Species like A. stephensi arehighly adaptable and are found to be very potent vectors of human malaria. Mosquitoeschoose the blood donor by odours and visual clues and can learn from experience!Human behaviour also plays a role and males are more frequently bitten.

The Anopheles genome- The genome of A. gambiae has now been cracked and identification of the mosquito genes involved in the parasite's transmission, resistance to insecticides, the mosquito's olfactory system, its immunity, its ability to digest blood, its choice of humans as a blood source etc should eventually lead to the development of ways to control the transmission of malaria by this vector.

The female mosquito has a specialised apparatus to penetrate the skin of its victim. At the end of the slender proboscis, there are two pairs of cutting stylets that slide against one another to slice through the skin. Once through the skin, the mosquito's proboscis begins probing for a tiny blood vessel. If it does not strike one on the first try, the mosquito will pull back slightly and try again at another angle through the same hole in the skin. Inside the proboscis are two hollow tubes, one that injects saliva into the microscopic woundand one that withdraws blood. The mosquito's saliva includes a combination of antihemostatic and

anti- inflammatory enzymes that disrupt the clotting process andinhibit the pain reaction (so that the victim is unaware of the bite!)

Mosquito need human blood because female mosquito lays 30-150 eggs every 2-3 days.Human blood is needed to nourish these eggs and Anopheles shows the most regularcycles of blood feeding and egg laying.Anopheles mosquitoes enter the house between 5 p.m. and 9.30 p.m. and again in early hours of morning. They start biting by late evening and the peak of biting activity is at midnight and early hours of morning. Mosquitoes can fly up to several kilometers! And they can reach far off places by taking shelter in motor vehicles, ships and aircraft. The average life span of a mosquito is 2-3 weeks. It can be longer in ideal living conditions.The adult mosquitoes hide themselves behind cupboards, clothes, curtains and other darkand cool corners during the day and come out to bite at night.

Anopheles mosquitoes breed in clean water collections. Therefore, breeding increases dramatically in the rainy season because many artificial water collections occur. During the rains, water collects in bottles, tins, tender coconut shells, buckets, tyres etc., that arethrown out in the open and these provide ample breeding ground. Also wells, ponds, water tanks, paddy fields etc., act as breeding grounds. Usually it takes about a week for the eggs to develop into adults.

Transmission of Malaria–

Vector mediated- Via the bites of female anopheles mosquito.Anopheles culicifacies is the predominant vector in rural areas. Anopheles stephensi is the predominant sub species in urban areas

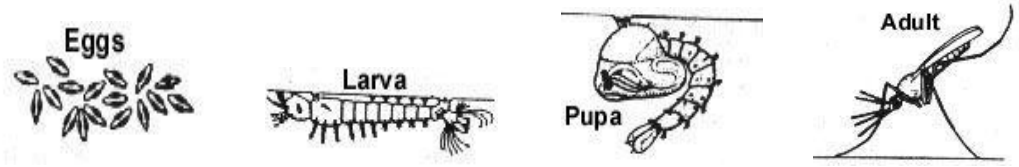
Blood transfusion (Transfusion malaria): This is fairly common in endemic areas. Following an attack of malaria, the donor may remain infective for years (1-3 years in *P. falciparum*, 3-4 years in *P. vivax*, and 15-50 years in *P. malariae*.) Most infections occur in cases of transfusion of blood stored for less than 5 days. Frozen plasma is not known to transmit malaria. The clinical features of transfusion malaria occur earlier and any patient who has received a transfusion three months prior to the febrile illness should be suspected to have malaria.In endemic areas, it is safe to administer full course of chloroquine to all recipients of blood transfusion. In this type of malaria, asexual forms are directly inoculated into the blood and pre-

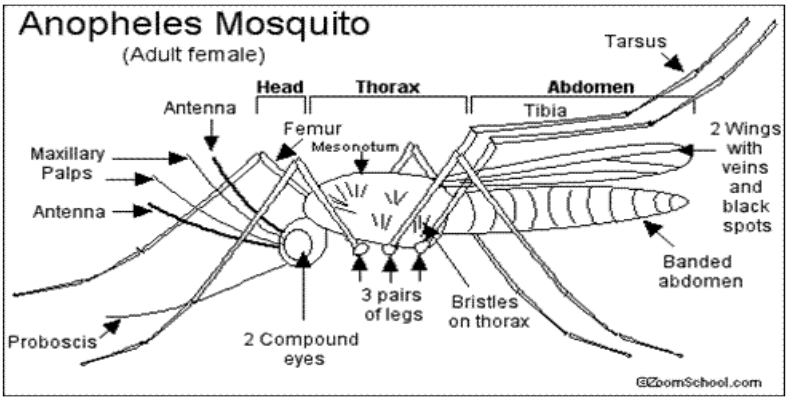
erythrocytic development of the parasite in the liver does not occur. Therefore, this type of malaria has a shorter incubation period and relapses do not occur. Therefore, treatment with primaquine for 5 (or14) days is not indicated.

If 1-5 million parasites are inoculated by i.v route then the incubation period is 8-21 days.If the same amount is inoculated by subcutaneous route or intramuscular route incubation period is 1-3 days.

Mother to the growing fetus <u>(Congenital malaria):</u> Intrauterine transmission of infection occurs when placenta becomes heavily infested with the parasites.

Needle stick injury: Accidental transmission can occur among drug addicts who share syringes and needles. (Therapeutic inoculation of malarial parasites, so as to induce fever, was a mode of treatment for neurosyphilis!)





The Malaria Parasite

Plasmodium species are apicomplexa and exhibit a heteroxenous life cycle involving a vertebrate host and an arthropod vector. Vertebrate hosts include: reptiles, birds, rodents, monkeys and humans.

Phylum: Protozoa **Subphylum:**Apicomplexa

Class: Sporozoa **Subclass:**Coccidia

Order: Coccidiida **Suborder:**Haemosporina

Family: Plasmodiidae

Genus: Plasmodia

Subgenera: Plasmodium, Laverania

Species (affecting man)

Quartan group: ***P. (Plasmodium) malariae***, *P. (P.) brasilianum*

Benign tertian group: ***P. (P.) vivax***, *P. (P.) cynomolgi, P. (P.) cynomolgibastianellii*

Malignant tertian group: ***P. (Laverania) falciparum***

Ovale group: ***P. (P.) ovale***, *P. (P.) simium*

Knowlesi group: *P. (P.) knowlesi*

Plasmodium species are generally host specific and vector specific in that each species will only infect a limited range of hosts and vectors.

P. malariae, P. vivax, P. falciparum and *P. ovale* are the most important types affecting man.

P. vivax is the commonest cause of human malaria in Central America, North Africa, southern and western Asia.

P. falciparum is the predominant species in Africa, New Guinea and Haiti.

P. falciparum is the cause for all mortality and most of the morbidity due to malaria.

Both *P. vivax* and *P. falciparum* are found in South America parts of Asia and Oceania.

P. ovale is restricted to western Africa.

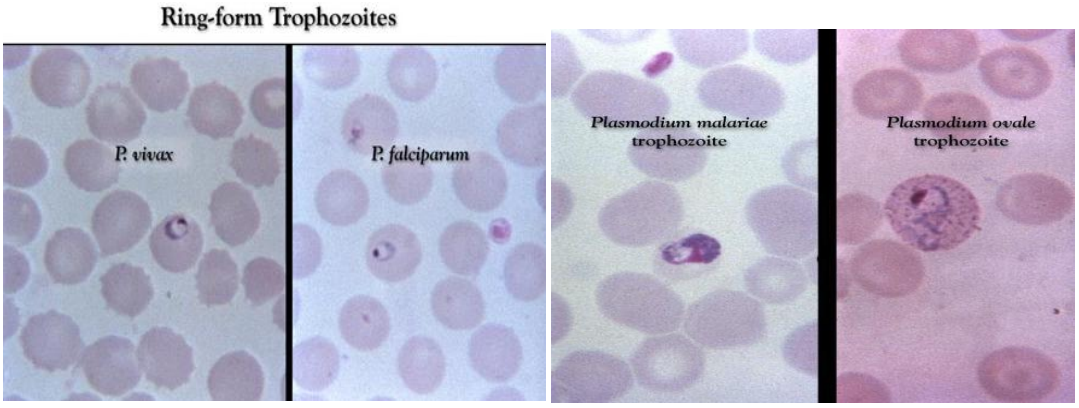
P. malariae is probably more wide spread than as made out to be in only a few pockets here and there.

Table 1: Characteristic feature of individual species

	P. falciparum	P. vivax	P. malariae	P. ovale
RBC Size	Not enlarged	Enlarged	Not enlarged	Enlarged
RBC Shape	Round, sometimes crenated	Round or oval,frequently bizarre	Round	Round or oval, often fimbriated
RBC Colour	Normal, but may become darker; may have purple rim	Normal to pale	Normal	Normal
Stippling	Maurer's spots, appear as large red spots, loops and clefts; up to 20	Schuffner's dots, appear as small red dots, numerous.	Ziemann's dots, few tiny dots, rarely detected	Schuffner's dots (James's dots). Numerous small red dots.
Pigment	Black or dark brown; in asexual forms as one or two masses; in gametocytes as about 12 rods	Seen as a haze of fine golden brown granules scattered through the cytoplasm	Black or brown coarse granules; scattered	Intermediate between P. vivax and P. malariae
Early trophozoite (ring)	Smallest, delicate; sometimes two chromatin dots; multiple rings commonly found	Relatively large; one chromatin dot, sometimes two; often two rings in one cell	Compact; one chromatin dot; single	Compact; one chromatin dot; single
Schizont	Medium size; compact; numerous chromatin masses; coarse pigments; rarely seen in peripheral blood	Large; amoeboid; numerous chromatin masses; fine pigments	Small; compact; few chromatin masses; coarse pigments	Medium size; compact; few chromatin masses; coarse pigments

Gametocyte	Crescent shaped, larger and slender; central chromatin	Spherical; compact	Similar to P. vivax, but smaller and less numerous	Like P. vivax, but smaller
Tissue schizogony	8 - 25 days	8 - 27 days	9 - 17 days	15 - 30 days
Erythrocytic phase	48 hours	48 hours	48 hours	72 hours
Red cells affected	All	Reticulocytes	Reticulocytes	Mature RBC's
Merozoites per schizont	8 - 32	12 - 24	4 - 16	6 - 12
Relapse from persistent liver forms	No	Yes	Yes	No, but blood forms can persist up to 30 years
Drug resistance	Yes	Yes	No	No

Species Appearance (Trophozoites)



Structure of Malarial parasite

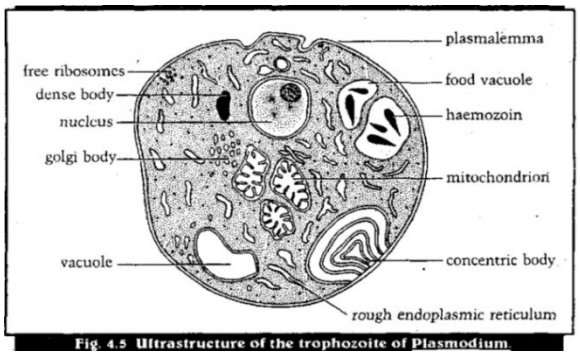
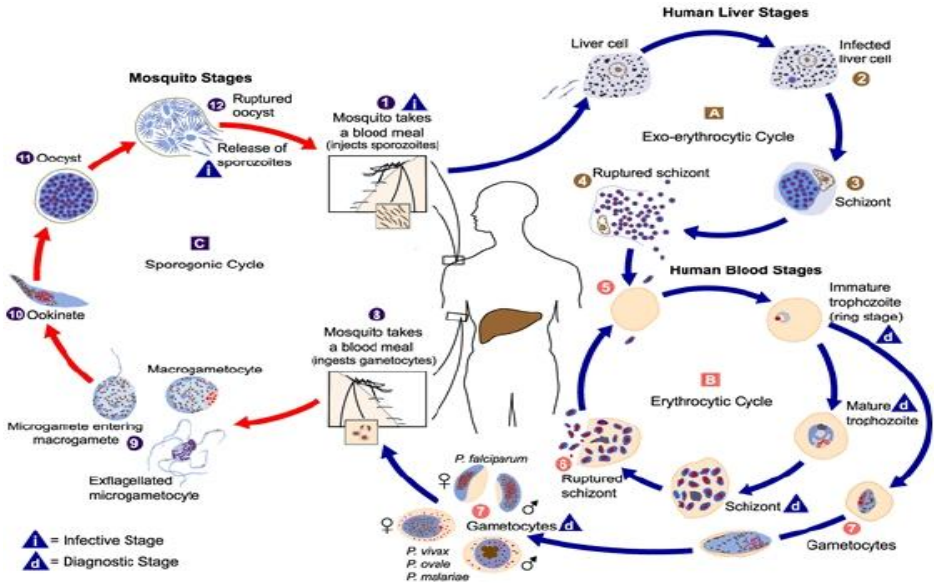


Fig. 4.5 Ultrastructure of the trophozoite of Plasmodium.

LIFE CYCLE :

The parasite is injected with the saliva during the anopheline mosquito feeding and first undergoes a round of merogony in the liver followed by multiple rounds of merogony in the erythrocytes. Gametogony begins within the erythrocytes of the vertebrate host and is completed within the mosquito where sporogony takes place. This life cycle exhibits the general features of other apicomplexan parasites characterized by asexual replication and the formation of invasive stages with typical apical organelles.



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IN MAN:

Liver Stage : **Pre-erythrocytic schizogony** : Human infection is initiated when **sporozoites** are injected with the saliva during mosquito feeding. The sporozoites enter the circulatory system and within 30-60 minutes will invade a liver cell. Host cell entry, as in all apicomplexa, is facilitated by the apical organelles. After invading the hepatocyte, the parasite undergoes an asexual replication. This replicative stage is often called **exoerythrocytic (or pre-erythrocytic) schizogony**. Schizogony refers to a replicative process in which the parasite undergoes multiple rounds of nuclear division without cytoplasmic division followed by a budding, or segmentation, to form progeny. The progeny, called merozoites, are released into the circulatory system following rupture of the host hepatocyte.

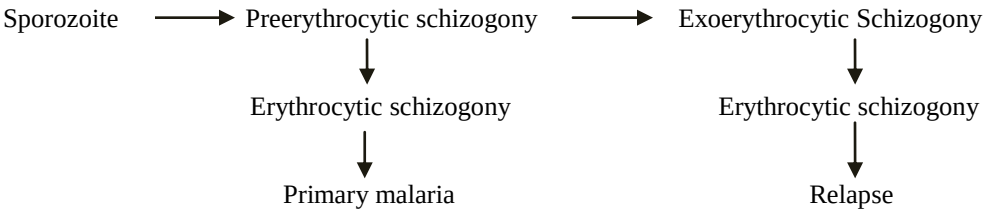
Exoerythrocytic schizogony and prepatent and incubation periods

	P. falciparum	*P. vivax*	*P. ovale*	*P. malariae*
Prepatent period (days)	6-9	8-12	10-14	15-18
Incubation period (days)	7-14	12-17	16-18	18-40
Merozoite maturation (days)	5-7	6-8	9	12-16
Merozoites produced	40,000	10,000	15,000	2000

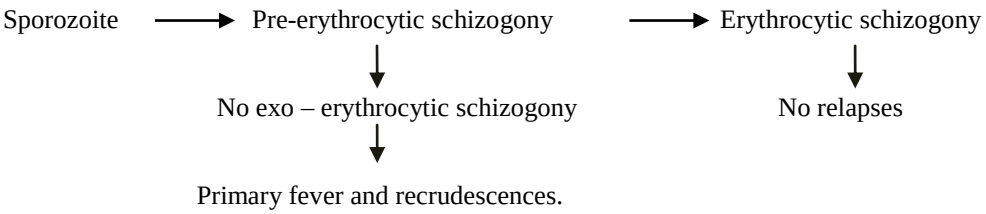
The time taken for the completion of the tissue phase is variable, depending on the infecting species; this interval is called as pre-patent period. In *P. vivax* and *P. ovale* some of the sporozoites do not immediately undergo asexual replication, but enter a dormant phase known as the **hypnozoite**. This hypnozoite can reactivate and undergo schizogony at a later time resulting in a relapse .Such parasites are responsible for the relapse of tertian malaria that may occur upto 2 years after the initial infection. **Relapse** has a specific meaning in regards to malaria and refers to the reactivation of the infection via hypnozoites.

Recrudescence is when parasitemia falls below detectable levels and later increases to patent parasitemia. Interestingly, strains isolated from temperate regions tend to exhibit a longer latent period between the primary infection and the first relapse than strains from tropical regions with continuous transmission

Tissue phase of P. vivax, P. malariae and P. ovale.



Tissue phase of P. falciparum malaria



Blood Stage :Erythrocytic schizogongy **: Merozoites**(mero = separate) released from the infected liver cells invade erythrocytes. The merozoites recognize specific proteins on the surface of the erythrocyte and actively invade the cell in a manner similar to other apicomplexan parasites. After entering the erythrocyte the parasite undergoes a trophic period followed by an asexual replication. The young trophozoite is often called a **ring form** due to its morphology in Geimsa-stained blood smears. As the parasite increases in size this 'ring' morphology disappears and it is called a **trophozoite**(trophos = nourish). During the trophic period the parasite ingests the host cell cytoplasm and breaks down the hemoglobin into amino acids. A by-product of the hemoglobin digestion is the malaria pigment, or hemozoin. These golden-brown to black granules have been long recognized as a distinctive feature of blood-stage parasites.

Nuclear division marks the end of the trophozoite stage and the beginning of the **schizont(**=split) stage. Erythrocytic schizogony consists of 3-5 rounds (depending on species) of nuclear replication followed by a budding process. Late stage schizonts in which the individual merozoites become discernable are called **segmenters**. The host erythrocyte ruptures and releases the merozoites. These merozoites invade new erythrocytes and initiate another round of schizogony. The intra erythrocytic cycle takes about 48 hours in P. vivax, P. ovale and P. falciparum infections .The 72 hour periodicity in *P. malariae* is due to its slower growth and maturation during blood-stage schizogony . In P. vivax malaria, the young red blood cells are predominantly infected, while in P. falciparum malaria, red blood cells of all ages are affected. Thus the infective load and severity of infection are more in case of P. falciparum malaria.

The blood-stage parasites within a host usually undergo a synchronous schizogonyand the merozoites are released at approximately the same time of the day. The simultaneous rupture of the infected erythrocytes and the concomitant release of antigens, Tumor Necrosis Factor , cytokines and waste products accounts for the intermittent fever paroxysms associated with malaria. Blood stage schizogony in *P. falciparum* differs from the other human malarial parasites in that trophozoite- and schizont-infected erythrocytes adhere to capillary endothelial cells and are not found in the peripheral circulation. This sequestration is associated with cerebral malaria.

Sexual Stage. As an alternative to schizogony some of the parasites will undergo a sexual cycle and terminally differentiate into either **micro- or macrogametocytes**. Male gametocytes are called Microgamete and female gametocytes are called Macrogametes The factors involved in the induction of gametocytogenesis are not known. However, commitment to the sexual stage occurs during the asexual erythrocytic cycle that immediately precedes gametocyte formations. Daughter merozoites from this schizont will develop into either all asexual forms or all sexual forms. Mature gametocytes appear in the peripheral blood after a variable period and enter the mosquito when it bites an infected individual. (5th day of primary attack in P. vivax and P. ovale, and thereafter become more numerous; appear at about 5 - 23 days after primary attack in P. malariae; appear after 8 -11 days of the primary attack in P. falciparum, rising in number until 3 weeks and falling thereafter, but may circulate for several weeks).Gametocytes do not cause any febrile reaction in human host. The individual who harbors gametocytes is known as 'carrier'. Gametocytes do not cause pathology in the human host and will disappear from the circulation if not taken up by a mosquito.

IN MOSQUITO:

Gametogenesis, or the formation of **micro-** and **macrogametes**, is induced when the gametocytes are ingested by a mosquito. The asexual forms which are ingested die off immediately.After ingestion by the mosquito, the microgametocyte undergoes three rounds of nuclear replication. These eight nuclei then become associated with flagella that emerge from the body of the microgametocyte. This process is readily observable by light microscopy due to the thrashing flagella and is called exflagellation. The macrogametocytes mature into macrogametes. However, at the morphological level this is much less dramatic than the exflagellation exhibited by the microgametocytes.

Exflagellation occurs spontaneously when infected blood is exposed to air. Critical factors involved in the induction of this gametogenesis are a decrease in temperature, a decrease in the dissolved carbon dioxide and the subsequent increase in pH to above 8.0 This somewhat mimics the environmental changes experienced by the gametocytes in that there will be a change to ambient temperature and the gut of the

mosquito exhibits a pH of approximately 7.8 as compared to a pH of 7.4 for blood. In addition, a mosquito-derived exflagellation factor (MEF) has also been described and identified as xanthurenic acid, a metabolite from insects. Xanthurenic acid lowers the permissive pH for exflagellation to below 8.0 and is possibly a biological cue for the parasite to undergo gametogenesis

Gametogenesis/Exflagellation

- occurs spontaneously after exposure to air
    - ↓ temperature (2-3^{o}C)
    - ↓ pCO_2
    - ↑ pH (8-8.3)
- Mosquito-derived ExflagellationFactor lowers permissive pH
- MEF = xanthurenic acid

The highly mobile microgametes will seek out and fuse with a macrogamete. Within 12-24 hours the resulting **zygote** develops into an **ookinete**. The ookinete is a motile invasive stage which will transverse both the peritrophic matrix and the midgut epithelium of the mosquito. Transversing the midgut epithelium involves invading and exiting several epithelial cells before emerging on the basal side of the epithelium. The invasion process is similar to other apicomplexa except that the ookinete does not have rhoptries and does not form a parasitophorous vacuole after invading the host cell.

Sporogony : After reaching the extracellular space between the epithelial cells and the basal lamina, the ookinete develops into an **oocyst**. The oocysts undergo an asexual replication, called sporogony, which culminates in the production of several thousand **sporozoites**. This generally takes 10-28 days depending on species and temperature and thereafter the mosquito remains infective for 1 - 2 months. Upon maturation the oocyst ruptures and releases the sporozoites which cross the basal lamina into the hemocoel(body cavity) of the mosquito.

These sporozoites are motile and have an ability to specifically recognize the salivary glands. After finding the salivary glands the sporozoites will invade and transverse the salivary gland epithelial cells and come to lie within its lumen. Some of these sporozoites will be expelled into the vertebrate host as the mosquito takes a blood meal, and thus reinitiate the infection in the vertebrate host. Although the hemocoel and salivary gland sporozoites are morphologically similar, they are functionally distinct. Salivary gland sporozoites efficiently invade liver cells, but cannot re-invade the salivary glands, whereas the hemocoelsporozoites are inefficient at invading liver cells.

Host factors:

Some patients are at special risk for developing malaria. These include patients with no immunity against malaria – infants and unexposed individuals and patients who have lost the acquired immunity like pregnant ladies, elderly and people who have left the area of transmission.

1. People of West African origin are strikingly non-susceptible to P. vivax infections. It has been found that P. vivax merozoites penetrate the red cells after binding to the Fya and Fyb receptors, which are the Duffy blood group antigen alleles.

2. The sickle cell trait (heterozygous state) has been found to confer protection against complications of P. falciparum malaria.

3. Hemoglobin F also has protective effects against severe malaria. In beta thalassemia,fetal hemoglobin levels are high.

4. In Melanasianovalocytosis, the rigid membrane of the red cells prevents entry of the parasites.

Pathogenesis

Pathology associated with all malarial species is related to the rupture of infected erythrocytes and the release of parasite material and metabolites, hemozoin (i.e, malaria pigment) and cellular debris. There is

an increased activity of the reticuloendothelial system, particularly in the liver and spleen and thus their enlargement, as evidenced by macrophages with ingested infected and normal erythrocytes and hemozoin.

Pre-erythrocytic schizogony: Sporozoites reach the liver in 30-40 minutes by brisk motility conferred by Circum Sporozoite Protein (CSP). Approximately 8-15 (up to 100) sporozoites are injected and therefore only a few hepatocytes are infected, therefore this stage of the infection causes no symptoms (rarely however a prodromal illness characterised by vague aches and pains, headache, nausea etc. may be present). Recent evidence indicates that sporozoites pass through several hepatocytes before invasion. The co-receptor on sporozoites for invasion involves, in part, the thrombospondin domains on the circumsporozoite protein and on thrombospondin-related adhesive protein (TRAP). These domains bind specifically to heparin sulfate proteoglycans on hepatocytes in the region in apposition to sinusoidal endothelium and Kuppfer cells. Within the hepatocyte, each sporozoite divides into 10000-30000 merozoites.

Erythrocytic schizogony: The sequence of invasion is similar for all Plasmodium spp. The merozoite first attaches to red cells. In P. falciparum, Erythrocyte Binding Antigen 175 and Merozoite Surface Protein 1, 2 with sialoglycoproteins have been identified as the ligands and in P. vivax, Duffy antigen on RBC is the site of binding. After the attachment to the red cell, the merozoitere orientates itself so that apposition of apical end occurs. This is followed by localized invagination and interiorization of the merozoite. The entire process is completed in 30 seconds.

The parasite has complex metabolic processes: It utilises amino acids from hemoglobin and detoxifies heme; pLDH, Plasmodium aldolase have been identified as enzymes of anerobic glycolysis. It has been found that the parasites increase the permeability of RBC to get nutrients, yet maintain the RBC structure for 48 hours. strengthened by RESA, allowing the immature parasite to survive. At the end, RBC ruptures and each schizont releases 6-36 merozoites

The P. falciparum has better skills for attack and can enter most RBCs, has plenty of redundant receptors and pathways and hence higher parasitemia. It also has better skills for survival: the parasite constantly

changes itself with 2% antigenic variation. Cerebral malaria neurological manifestations are believed to be due to the sequestration of the infected erythrocytes in the cerebral microvasculature. Sequestration refers to the cytoadherence of trophozoite- and schizont-infected erythrocytes to endothelial cells of deep vascular beds in vital organs, especially brain, lung, gut, heart and placenta. The major advantage is the avoidance of the spleen and the subsequent elimination of infected erythrocytes. In addition, the low oxygen tensions in the deep tissues provide a better metabolic environment. Sequestration of RBC's containing mature forms of the parasite in vital organs interferes with microcirculatory flow and metabolism.

Cytoadherence appears to be mediated by the electron-dense protuberances on the surface of the infected erythrocyte. These 'knobs' are expressed during the trophozoite and schizont stages and are formed as a result of parasite proteins exported to the erythrocyte membrane.

***Pf*EMP-1**
• exposed on surface of infected erythrocytes • binds to potential host receptors • member of *var* gene family

The molecular mechanisms of cytoadherence involve receptor-ligand interactions. Proteins expressed on the surface of the infected erythrocyte (ligand) will bind to proteins expressed on the surface of the endothelial cells (receptor). *Pf*EMP-1 (erythrocyte membrane protein) is a parasite protein which has been implicated as the cytoadherence ligand (Box). In constrast to the usually highly conserved nature of receptor-ligand interactions, *Pf*EMP-1 is a member of a highly variable (= *var*) gene family with 40-50 different genes. Several host proteins which possibly function as receptors have been identified (see box below). Many of these host proteins function in cell-cell interactions and are involved in cellular adhesion. Several studies have indicated that the expression of different *Pf*EMP-1 genes is correlated with different receptor-binding phenotypes. This antigenic variation associated with the surface exposed *Pf*EMP-1 allows the parasite to evade the immune system. However, the cytoadherence function is preserved through its ability to recognize multiple receptors. This antigenic variation may also account for different disease outcomes. For example, intercellular adhesion molecule-1 (ICAM-1) is usually implicated in cerebral pathology.

Possible Receptors

• CD36
• Ig Super-family
  ○ ICAM-1
  ○ VCAM-1
  ○ PE-CAM-1
• E-selectin
• thrombospondin (TSP)
• chondroitin sulfate A
• Rosetting Receptors
  ○ CR-1
  ○ glycosaminoglycan

Rosetting is adherence of parasitised red cells with uninfected red cells. It is independent of venular cytoadherence and exhibits 5 times stronger adhesion. Rosetting causes higher microvascular obstruction and is associated with cerebral malaria (cytoadherence with other vital organ damage). Rosetting reduces blood flow, encourages cytoadherence to endothelium, enhances anerobic glycolysis and reduces the pH.

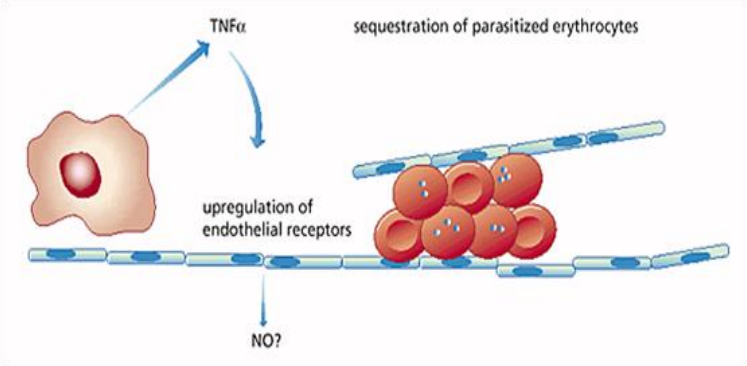
Early observations of the pathology of cerebral malaria suggested a relationship between large numbers of infected erythrocytes in the microvasculature and the development of the syndrome . Unbridled cytoadherence-rosetting-sequestration results in poor tissue perfusion, organ dysfunction, anerobic glycolysis in tissues and lactic acidosis, malfunctioning of dendritic cells and T cells due to CD36 binding. The hypoxia and metabolic effects would then cause the coma and subsequent death. However, there are some problems with the sequestration hypothesis:

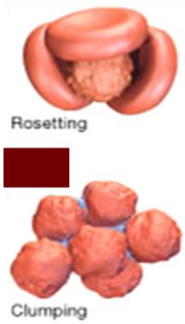
- the coma associated with cerebral malaria is rapidly reversible upon treatment,
- a high percentage of survivors have no permanant neurological complications,

- a lack of ischemic damage (rules out hypoxia as major mechanism),
- sequestration also occurs in non-cerebral malaria.

Because of these problems others have suggested that the coma is mediated by short-lived molecules that affect cerebral function. Possible host mediators include cytokines, such as TNF-a, or nitric oxide. In this cytokine theory, malarial antigens would stimulate TNF-a which could then induce nitric oxide or have other pathological effects. Nitric oxide is known to affect neuronal function and it could also lead to intracranial hypertension through its vasodilator activity. It is unlikely, though, that the systemic release of cytokines would cause coma and one needs to also postulate that release of these mediators in the brain would lead to high local concentrations. In addition, there is minimal lymphocyte infiltration or inflammation associated with the blocked capillaries.

The sequestration hypothesis and cytokine theory for the pathophysiology of cerebral malaria are not mutually exclusive, and both phenomenon are likely to be involved. For example, parasite exo-antigens, which are released at erythrocyte rupture, are known to stimulate macrophages to secrete TNF-alpha. TNF-alpha is known to upregulate the expression of adhesion molecules such as ICAM-1 on the surface of brain endothelial cells. This would lead to increase binding of infected erythrocytes and amplify the effects whether they are due to vascular blockage, soluble mediators, metabolic effects, or a combination.





Immunological response in malaria :

Development of immunity in malaria is manifested by tolerance to infection (cessation of clinical phenomenon despite parasitaemia) which is the result of active immunity (both cellular and humoral).The

phagocytic activity of the cells of the reticuloendothelial system (particularly those in the spleen and the liver) helps in the development of immunity to malarial infection. The parasites are destroyed and kept at subclinical levels. Hence it has been suggested that immunity in malaria depends on a persistent latent infection , known as infection immunity or premunition. The antiparasitic defense mechanisms of the host are effective only against the asexual erythrocytic parasites (mature schizonts and free merozoites) but not against the gametocytes and the exo-erythrocytic forms.

It is of two types:

1. Innate immunity.

2. Acquired immunity.

INNATE IMMUNITY: is seen in

a) Sickle cell anaemia

b) Abnormal haemoglobins- HbS, HbE, HbC

c) Hereditary spherocytosis

d) Thalessemia

e) G6PD deficiency

f) MHC class 1 and 2 / HLA BW53 and DRB 1/ 1302

ACQUIRED IMMUNITY: is of two types

a) Humoral

b) Cell mediated

HUMORAL IMMUNITY

There is increase in immunoglobulins. Majority are IgG and non specific stimulants.These instead of being protective may infact interfere with cell mediated immunity[6]. Both IgG and IgM are increased markedly. But only 6-11% 0f total immunoglobulins are specific for malarial antigens[7].

The spleen plays an important role in effectively curtailing the parasitemia by removal of parasitized RBC's and free merozoites by antibody mediated phagocytosis, entrapment ofinfected erythrocytes having reduced deformability and damaging intracellular parasitesby oxygen radicals and oxygen dependant factors generated by splenic macrophages.

CELL MEDIATED IMMUNITY

It plays an important role in total Immunity of the host. It leads to T cell activation as studied by cellular proliferation, lymphocyte secretion, appearance of T cell dependant isotopes of antibody, increase in IL-2 levels. Both CD4 and CD8 cells increase. There isan inversion of CD4 and CD8 ratio, due to disproportionate increase in CD8 levels.Protection against reinfection with malaria could be achieved by T cells dependant mechanisms even in the absence of antibodies [7]. T cells participate in MHC non restricted cytotoxicity and help in destruction of RBC's.

CLINICAL MANIFESTATIONS

The pathology and clinical manifestations associated with malaria are almost exclusively due to the asexual erythrocytic stage parasites. Tissue schizonts and gametocytes cause little, if any, pathology. *Plasmodium* infection causes an acute febrile illness which is most notable for its periodic fever paroxysms occuring at either 48 or 72 hour intervals. The severity of the attack depends on the *Plasmodium* species as well as other circumstances such as the state of immunity and the general health and nutritional status of the infected individual. Malaria is a chronic disease which has a tendency to relapse or over months or even years.

Symptoms of malaria usually start to appear 10-15 days after the bite of an infected mosquito. The typical prepatent and incubation periods following sporozoite inoculation vary according to species (Table). The **prepatent period** is defined as the time between sporozoite inoculation and the appearance of parasites in the blood and represents the duration of the liver stage and the number of merozoites produced. **Incubation periods** tend to be a little longer and are defined as the time between sporozoite inoculation and the onset of symptoms. Sometimes the incubation periods can be prolonged for several months in *P. vivax, P. ovale,* and *P. malariae*. All four species can exhibit non-specific prodromal symptoms a few days before the first febrile attack. These prodromal symptoms are generally described as 'flu-like' and include: headache, slight fever, muscle pain, anorexia, nausea and lassitude. The symptoms tend to correlate with increasing numbers of parasites.

These prodromal symptoms will be followed by febrile attacks also known as the malarial paroxysms. These paroxysms will exhibit periodicities of 48 hours for *P. vivax, P. ovale*, and *P. falciparum*, and a 72-hour periodicity for *P. malariae.* Initially the periodicity of these paroxyms may be irregular as the broods of merozoites from different exoerythrocytic schizonts synchronize. This is especially true in *P. falciparum* which may not exhibit distinct paroxysms, but exhibit a continuous fever, daily attacks or irregular attacks (eg., 36-48 hour periodicity). Patients may also exhibit splenomegaly, hepatomegaly (slight jaundice), and hemolytic anemia during the period in which the malaria paroxysms occur.

Clinical manifestations fall into one of the following.

1. Uncomplicated acute malaria
2. Severe or complicated malaria.
3. Chronic malaria.
4. Tropical splenomegaly syndrome.

UNCOMPLICATED MALARIA: Acute febrile illness with QBC & peripheral smear positive for malaria. The classical features may be masked by early treatment.

Typical features:	**The Malarial Paroxysm**	
cold stage	hot stage	sweating stage
• feeling of intense cold • vigorous shivering • lasts 15-60 minutes	• intense heat • dry burning skin • throbbing headache • lasts 2-6 hours	• profuse sweating • declining temperature • exhausted and weak → sleep • lasts 2-4 hours

The malarial paroxysm (see Table) will usually last 4-8 hours and begins with a sudden onset of chills in which the patient experiences an intense feeling of cold despite having an elevated temperature. This is often referred to as the cold stage and is characterized by a vigorous shivering. Immediately following this cold stage is the hot stage. The patient feels an intense heat accompanied by severe headache. Fatigue, dizziness, anorexia, myalgia, and nausea will often be associated with the hot stage. Next a period of profuse sweating will ensue and the fever will start to decline. The patient is exhausted and weak and will usually fall asleep. Upon awakening the patient usually feels well, other than being tired, and does not exhibit symptoms until the onset of the next paroxysm

Each species of plasmodium causes a characteristic fever designated as follows:

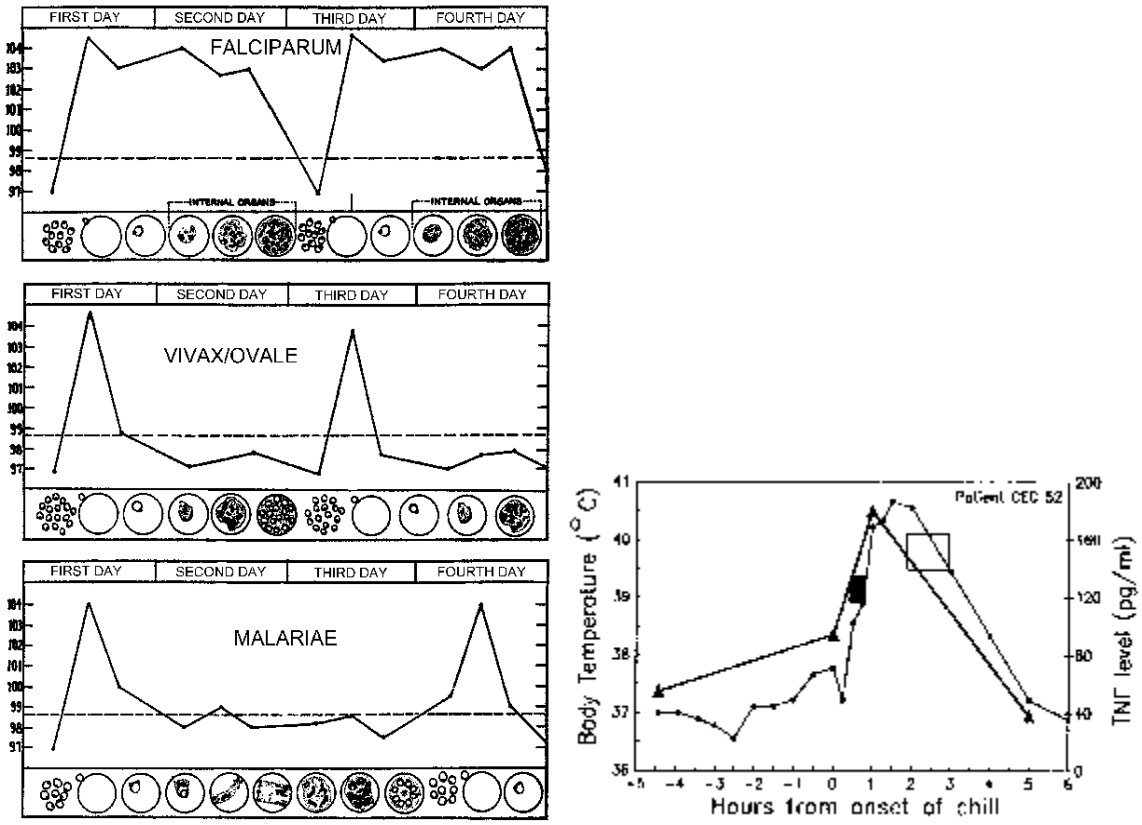
a) Plasmodium vivax -- fever recurs once every 48 hours = benign tertian malaria

b) Plasmodium falciparum-- malignant tertian malaria -- pattern may not be seen often and the paroxysms tend to be more frequent (Sub-tertian)

c) Plasmodium malariae -- relapses occur once every 72 hours = quartan malaria

d) Plasmodium ovale -- Ovale tertian malaria

e) Mixed Infection -- quotidian fever



A typical pattern of temperature (fever) in relation to blood-stage schizogony for the human malarial parasites. The fever paroxysm corresponds to the period of infected erythrocyte rupture and merozoite invasion. [108]

The periodicity of these paroxysms is due to the synchronous development of the malarial parasite within the human host. In other words, all of the parasites within a host are at approximately the same stage (ie, ring, trophozoite, schizont) as they proceed through schizogony. The malarial paroxysm corresponds to the rupture of the infected erythrocytes and the release of merozoites (Figure above). Studies in *P. vivax* have demonstrated a correlation between fever and serum TNF-α (tumor necrosis factor-alpha) levels (Figure right). Presumably antigens or toxins are released when the infected erythrocyte ruptures and lead to the production of TNF-α and the febrile attacks.

The severity of the paroxysms and duration of the symptoms varies according to species (see Table below). In general, the severity of the disease correlates with the average and maximum parasitemia exhibited by the

various species. *P. falciparum* can produce a severe and lethal infection, whereas the other species are rarely mortal. Patients infected with *P. vivax*, especially for the first time, can be quite ill. However, *P. vivax* rarely causes complications or results in death. On occassion severe malaria involving multiple organs has also been noted in *P. vivax* infections. Relapses to the activation of *P. vivax* hypnozoites can occur for several years. *P. ovale* is the most benign in that the paroxysms tend to be mild and of short duration and relapses seldom occur more than one year after the initial infection. *P. malariae* generally produces a mild disease, but the initial paroxysms can be moderate to severe. It is the most chronic, though, and recrudescences have been documented several decades after the initial infection. This chronicity is sometimes associated with renal complications, which are probably due to the deposition of antigen-antibody complexes in the glomeruli of the kidney. The malarial paroxysms will become less severe and irregular in periodicity as the host develops immunity. This immunity, however, is not a sterilizing immunity in that the infection persists longer than the symptoms and individuals can exhibit relapses or recrudescences or become reinfected. If untreated, all forms of malaria tend to be chronic.

Disease Severity and Duration

	vivax	**ovale**	**malariae**	**falciparum**
Initial Paraoxysm Severity	moderate to severe	mild	moderate to severe	severe
Average Parasitemia (mm^3)	20,000	9,000	6,000	50,000-500,000
Maximum Parasitemia (mm^3)	50,000	30,000	20,000	2,500,000
Symptom Duration	3-8+ weeks	2-3 weeks	3-24 weeks	2-3 weeks

Maximum Infection Duration (untreated)	5-8 years	12-20 months	20-50+ years	6-17 months
Anemia	++	+	++	++++
Complications			renal	cerebral

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SEVERE OR COMPLICATED MALARIA 8:

In contrast to the other three species, *P. falciparum* can produce serious disease with mortal consequences.

Complicated malaria(smear positive) : Clinically defined as

a. Those in coma and unable to localize a painful stimulus (Cerebral malaria)
b. Fully conscious but either prostrated (unable to maintain a sitting posture) or in respiratory distress (abnormally deep breathing with intercostals or subcostal recession)

According to WHO, the criteria for severe malaria are: A history of possible malaria exposure and no other cause for clinical illness or laboratory diagnosis of Plasmodium falciparum malaria and one or more of the following 8

Complicated malaria (WHO criteria)

1. Impaired consciousness (but arousable)
2. Prostration and extreme weakness.
3. Jaundice
4. Cerebral malaria (unarousable coma not attributable to any other cause in a patient with falciparum malaria)
5. Generalised convulsions

6. Normocytic anemia
7. Renal failure
8. Hypoglycemia
9. Fluid, electrolyte, acid base disturbances
10. Pulmonary oedema
11. Circulatory collapse and shock (algid malaria)
12. DIC
13. Hyperpyrexia
14. Hyperparasitemia
15. Malarial haemoglobinuria

Indicators of severe malaria and poor prognosis

Manifestation	Features
Initial World Health Organization criteria from 1990	
Cerebral malaria	Unrousable coma not attributable to any other cause, with a Glasgow Coma Scale score ≤ 9. Coma should persist for at least 30 min after a generalized convulsion
Severe anemia	Hematocrit <15% or hemoglobin < 50 g/l in the presence of parasite count >10 000/μl
Renal failure	Urine output <400 ml/24 hours in adults (<12 ml/kg/24 hours in children) and a serum creatinine>265 μmol/l (> 3.0 mg/dl) despite adequate volume repletion
Pulmonary edema and acute respiratory distress syndrome	The acute lung injury score is calculated on the basis of radiographic densities, severity of hypoxemia, and positive end-expiratory pressure 54
Hypoglycemia	Whole blood glucose concentration <2.2 mmol/l (<40 mg/dl)
Circulatory collapse (algid malaria)	Systolic blood pressure <70 mmHg in patients > 5 years of age (< 50

	mmHg in children aged 1–5 years), with cold clammy skin or a core-skin temperature difference >10°C
Abnormal bleeding and/or disseminated intravascular coagulation	Spontaneous bleeding from gums, nose, gastrointestinal tract, or laboratory evidence of disseminated intravascular coagulation
Repeated generalized convulsions	≥ 3 convulsions observed within 24 hours
Acidemia/acidosis	Arterial pH <7.25 or acidosis (plasma bicarbonate <15 mmol/l)
Macroscopic hemoglobinuria	Hemolysis not secondary to glucose-6-phosphate dehydrogenase deficiency
Added World Health Organization criteria from 2000 55	
Impaired consciousness	Rousable mental condition
Prostration or weakness	
Hyperparasitemia	> 5% parasitized erythrocytes or > 250 000 parasites/μl (in nonimmune individuals)
Hyperpyrexia	Core body temperature >40°C
Hyperbilirubinemia	Total bilirubin >43 μmol/l (> 2.5 mg/dl)

A study conducted by Krishnan A et.al in 2003 on Severe falciparum malaria: an important cause of multiple organ failure in Indian intensive care unit patients, concluded that Malaria is an important cause of multiple organ failure in India. Mortality rate is 6.4% when one or fewer organs fail but increases to 48.8% with failure of two or more organs. However, outcomes are better than for similar degrees of organ failure in sepsis [12].

A study conductd by Koulmann P et.al in 2003 on severe malaria in intensive care units suggested that, malaria requiring intensive care is characterized by failure of one or more organ systems and/or

development of several metabolic disorders secondary to the presence of Plasmodium faliciparum in the blood. Mortality rates associated with management of severe imported malaria in intensive care range from 10 to 30% [13].

A study conducted by Mishra S K et.al in 2007 on Prediction of outcome in adults with severe falciparum malaria: a new scoring system, suggested that MSA (malaria scoring in adults) is a simple and sensitive predictor. It can be administered rapidly and repeatedly to prognosticate the outcome of severe malaria in adults. It can help the treating doctor to assess the patient. The mortality was 2% for MSA 0-2; 10% for MSA 3-4, 40% for MSA 5-6 and 90% for MSA 7 or more. The sensitivity is 89.9% and positive predictive value is 94.1% [14].

CEREBRAL MALARIA [15]:

It is the most common complication and cause of death in severe Plasmodium falciparum infection. Central nervous system manifestations are fairly common in malaria and it could be due to not only severe falciparum infection but also high grade fever and anti-malarial drugs. Definition of cerebral malaria includes the presence of unarousable coma, exclusion of other encephalopathies and confirmation of Plasmodium falciparum infection.

Manifestation of cerebral malaria may be impaired consciousness, delirium, and abnormal neurological signs and focal or generalized convulsions [29]. In severe Falciparum malaria the neurological dysfunction can manifest suddenly following generalized convulsions or gradually over a period of time.

Pathophysiology [16]:

Clogging of cerebral microcirculation by the parasitized red cells because of development of knobs on their surface and increased cytoadherance properties as a result of which they tend to adhere to endothelium of capillaries and venules. Obstruction of the microcirculation leads to hypoxia and increased lactate production. Vascular permeability is also increased. About 80% of children with cerebral malaria have raised ICT, due to increased cerebral blood volume and biomass [16].

Mechanism of coma in cerebral malaria: It is not clearly known. Cytokines induce nitric oxide synthesis in leukocytes, smooth muscle cells, microglia and endothelium. Nitric oxide is a potent inhibitor of neurotransmission leading to coma.

Causes of neurological manifestations in malaria:

High grade fever

Antimalarial drugs like chloroquine, quinine, Mefloquine and halofantrine.

Hypoglycemia- due to severe malaria or quinine

Hyponatremia- due to excessive vomiting

Severe anemia and hypoxia.

Neurological signs in cerebral malaria [17]:

Unarousable coma, GCS <7/15

Mild neck stiffness may be seen.

Retinal haemorrhages are seen in 15%

Varieties of transient abnormalities of eye movements are seen – common is dysconjugate gaze.

Fixed jaw closure, bruxism.

Motor abnormalities such as decerebrate rigidity, decorticate rigidity and ophisthotonus may occur.

Neurological sequelae [17];

Psychosis (5.065%)

Cerebellar ataxia (4.72%)

Hemiplegia (1.68%)

Extra pyramidal rigidity (1.35%)

Peripheral neuropathy (1.01%)

Extra pyramidal rigidity with trismus (0.33%)

6th cranial nerve palsy (0.33%)

Hematological complications in Malaria

- Hematological changes are very common in malaria.
- These include :-
- Anaemia one of the most common complication particularly due to P.Falciparum infection.
- Leucopenia or Leucocytosis
- Thrombocytopenia
- DIC
- Hematological complication while seen both in P.Falciparum and P.Vivax malaria can become a serious and life threatening in Falciparum malaria. The reason for these being high level of parasitemia associated with P.Falciparum .The severity of infection and the haematological complication is modulated by immune status of the host,nutritional factors, and inter current infection and genetic as well as time to presentation and duration of the illness. Pathophysiological mechanisms contributing to hematological changes are both complex and multi- factorial which includes :-

- Activation of immune complex system by antigens released by the parasites and damage to the hematological cells.
- Rupture of red cells due to multiplying parasites inside the blood cells.
- Reversible bone marrow suppression, hypersplenism and hyperplasia of the reticulo endothelial systems.

A study conducted by Lathia TB et al on hematological parameters discriminate malaria from non malarious acute febrile illness in the tropics from Mahatma Gandhi Institute of Medical Sciences, Maharashtra suggested that low hemoglobin and low platelet count are the two hematological variables that increase the probability of malaria, by factor of 1.95 and 5.04 respectively. These two variables also emerge useful when used in combination (Likelihood ratio 2.77). The 95% confidence interval for RDW however crosses one, which implies measurement of this parameter to be less precise [18]

HASAN AM et al in their study "decreased erythropoietin response in plasmodium Falciparum malaria – associated anaemia" have shown that there is inadequate production of erythropoietin in patients with falciparum malaria [19].

Sharma SK et al in their study of 30 cases of falciparum malaria about hematological and coagulation profile in acute falciparum malaria observed severe anaemia in 10% 0f cases and it was associated with 100% mortality [20].

The pathogenesis of anemia in malaria is multifactorial. A complex chain of pathogenetic processes involving mechanical destruction of parasitized RBC's, marrow suppression, ineffective erythropoiesis and accelerated immune destruction of nonparasitized RBC's have been implicated. [21] Thrombocytopenia is a common observation in falciparum malaria with spontaneous recovery on treatment. Both leucopenia [22] and leukocytosis [23] have been described in malaria.

Severe malaria is also associated with reduced deformability of the uninfected erythrocytes.[24] This compromises their passage through the partially obstructed capillaries and venules and shortens RBC survival. Anaemia results from accelerated RBC destruction and removal by the spleen in conjuction with ineffective erythropoiesis [19,20]. In severe malaria both infected and uninfected RBCs, show reduced deformability which correlates with prognosis and development of anemia [25]. In non-immune individuals and in areas with unstable transmission, anemia can develop rapidly and transfusion is often required [26,27,28]

Anemia is a common consequence of antimalarial drug resistance, which results in repeated or continued infection [29]. Slight coagulation abnormalities are common in falciparum malaria and mild

thrombocytopenia is usual. However <5% of patients with severe malaria have significant bleeding with evidence of DIC.

The term Malarial Haematopathy attempts to describe the involvement of one or more haematopoietic cell lines and includes the endothelial dysfunction that can cause thrombotic microangiophthy that may evolve in to consumptive coagulopathy

RDW is an index of variation in red cell volume within the red cell population. It is a parameter provided by various cytometers. Mathematically it is coefficient of variation i.e.

$$\text{RDW} = \frac{\text{Standard Deviation of red cell volume}}{\text{Mean red cell volume}} \times 100$$

Red cell population with higher than normal RDW's are termed hetergenous and those with normal RDW homogenous. Increase in number of reticulocytes causes increased RDW. In some cases, increased RDW is the first change to appear as in early iron deficiency anemia as there are few microcytes in blood at this stage and mean RBC volume is hence normal. Normal range is 11.5 to 14.5. An increased RDW indicates anisocytosis. Increased RDW and intraerythrcytic Hb concentration were demonstrated in malaria. The increased RDW correlated with increased % of macrocytes. RDW increases when erythropoiesis is stimulated

LEUCOPENIA OR LEUCOCYTOSIS

Total leukocyte count is usually normal, however leukocytosis can ccur especially when associated with pernicious malaria and superadded bacterial infections [30,31,32]

Sharma SK et al in their study of 30 cases of falciparum malaria concluded that leucocytosis is encountered when associated bacterial infection and pernicious syndromes exist [20]

Sen et al in their study concluded that leucopenia and neutropenia may be explained by bone marrow or by a state of hypersplenism although total neutrophil pool has been reported to be increased in malaria. The normal or decreased neutrophil count has been explained on the basis of expanded marginal pool, particularly spleen. [33]

In Kenyan studies leukocytosis was associated with both severity and mortality in children with falciparum malaria irrespective of bacteraemia. Increase in number of atypical lymphocytes has been reported in acute falciparum infection at times leading to false positive serological tests like widal titres.[34,35]

THROMBOCYTOPENIA

Thrombocytopenia is seen in both complicated and uncomplicated malaria. Thrombocytopenia is a common feature of acute malaria and occurs in both P. falciparum and P. vivax infections regardless of the severity of infection.[34,35,36] The absence of normal quantity of platelets on a peripheral smear in a case of fever is often a clue to the presence of malaria. Immune mediated lysis, sequestration in the spleen and a dyspoietic process in the marrow with diminished platelet production have all been postulated. Abnormalities in platelet structure and function have been described as a consequence of malaria, and in rare instances platelets can be invaded by malarial parasites themselves.[37,38] Patients with malaria can also have platelet function abnormalities.[39]

A study conducted by Jain M.et.al in 2005 on Comparative study of microscopic detection methods and haematological changes in malaria, observed that Anaemia was present in 66 (94.28%) samples of which 37 (56.06%) were Plasmodium falciparum, 21 (31.81%) were Plasmodium vivax and 8 (12.12%) had mixed infection (Plasmodium falciparum and Plasmodium vivax). 35 (50%) cases showed normocytic normochromic anaemia. Majority of the samples showed normal total and differential leukocyte count.

Thrombocytopenia was found in 49 (70%) samples of which 33 (67.34%) were Plasmodium falciparum [40].

In the study by Sharma. K. et al Thrombocytopenia was present in as high as 90% of patients, [20]

Horstman et al found thrombocytopenia in 85% of P.Falciparum and 72% of P.Vivax patients respectively.[41]

A Hospital based study in Saudi Arabia showed that thrombocytopenia is more commonly found than anaemia in malaria.

Sen et al in their study concluded that transient hypoplasia of bone marrow may be one of the mechanism for thrombocytopenia [25].

Sorabjee J S in his study concluded that absence of normal quantity of platelets in case of fever is often a clue to presence of malaria [42].

Malhotra et al in their study concluded that platelet count and parasitic load are inversely proportional and thrombocytopenia may not always indicate DIC.[43]

S. Ladhani et al in their study of 1969 cases of malaria concluded that though thrombocytopenia was common in malaria it was not associated with adverse outcome [44].

UM Jadhav et al in their study concluded that presence of thrombocytopenia is not a distinguishing feature between vivax and falciparum malaria. Thrombocytopenia of <20000/microL can occur in vivax though statistically more significant with falciparum malaria [45].

Thrombocytopenia is generally unrelated to clinical severity but the degree of thrombocytopenia co-related with the size of the spleen . Thrombocytopenia usually resolves spontaneously once the infection subsides. The pathogenesis of thrombocytopenia is thought to be similar to that of anaemia and they often co-exists .Various study have shown that anaemia and thrombocytopenia occur simultaneously and subside gradually with therapy and clearance of parasitemia.

The factors involved in pathogenesis of thrombocytopenia include

1) Hypersplenism and splenic pooling of parasites.

2) Hypersplasia of reticulo endothelial cells and increased phagocyte destruction.

3) Destruction of platelets bound by immune complexes by the reticulo endothelial system.

4) And rarely disseminated intra vascular coagulation.

Immunological basis for thrombocytopenia [46,47]

The low platelet count emerged as the strongest predictor of malaria. In a study on over two thousand patients with fever, Erhart et al [48] reported platelet count of less than 1,50,000 increases the likelihood of malaria by 12-15 times. Various other studies have also found thrombocytopenia to be commonly associated with malaria [49,50] which resolves after therapy[51] . The suggested mechanisms for thrombocytopenia include disseminated intravascular coagulation, or excessive removal of platelets by reticulo-endothelial system.[52] Anti-Platelet IgG has also been implicated in the pathogenesis of thrombocytopenia. [42] Thrombocytopenic malaria, in contrast to the non-thrombocytopenic variety correlates with a higher degree of parasitemia and increased cytokine production.[53]

A Study conducted by Koltas et.al in 2007 on Supportive presumptive diagnosis of Plasmodium vivax malaria. Thrombocytopenia and red cell distribution width. Suggested that routinely used laboratory findings such as low hemoglobin, leukocyte or platelet counts and especially high red cell distribution width values could present a more supportive clue in the diagnosis of vivid malaria in endemic areas [54.]

Platelets are thought to be passively absorbed by the malarial antigen which then bind to Immunoglobulin molecules . These antibody coated platelets are then cleared by phagocytosis in the spleen .

Towze et al in their series of patients infected with malaria showed that there was an inverse relationship between the platelet counts and the platelet anti body level.[33] This was supposed to be the cause for thrombocytopenia however loreesuwan et al showed that there was no relationalship between platelet count and the platelet antibody level [56] It is possible that platelet bound immunoglobulin is a qualitative recognition trigger for splenic removal of platelet that the threshold is lowered in patients with malaria.

A study conducted by Jadhav U.M et al on "Thrombocytopenia in Malaria-Correlation with type and severity "A total of 1565 subjects, either hospitalized or treated on an out patient basis over a period of three years . 590 subjects had P. falciparum malaria and two subjects had mixed parasitemia of P. vivax and P. falciparum malaria. Platelet count less than 1,50, 000/dl was noted in 79.4% cases.[45]

Falciparum malaria presents with protean manifestations and is associated with a variety of complications and has a high mortality. Thrombocytopenia is a common feature of acute malaria and occurs in both P. falciparum and P. vivax infections regardless of the severity of infection. The absence of the normal quantity of platelets on a peripheral smear in a case of fever is often a clue to the presence of malaria. Thrombocytopenia is rarely accompanied by clinical bleeding or biochemical evidence of DIC. Platelet counts can fall to below 25,000/cmm but this is uncommon. Platelet counts rise rapidly with recovery.

The prevalence of thrombocytopenia highlights the fact that a persistent normal platelet count is unlikely in the laboratory findings of malaria.[2] Maximum thrombocytopenia occurred on the fifth or sixth day of infection, and gradually returned to normal within 5-7 days after parasitemia ceased [57,58].The mechanism of thrombocytopenia in malaria could be due to peripheral destruction and consumption by DIC. This must be considered in the context that very low platelet counts can be transient in the course of malaria illness. Clinical bleeding in severe malaria is not a common feature and occurs in less than 5-10% of individuals with severe disease [18,59] . Platelet and fibrin deposition are rarely seen in the capillaries of patients at postmortem and despite numerous studies indicating elevated levels of fibrin degradation products, clinical DIC is rare. Also, thrombocytopenia, per se cannot be a distinguishing feature in a particular case of malaria, although there is a statistical significant difference in the prevalence and severity of thrombocytopenia between the two types of malaria.

The mechanism of thrombocytopenia in malaria is uncertain. Immune-mediated lysis, sequestration in the spleen and a dyspoietic process in the marrow with diminished platelet production have all been postulated. Abnormalities in platelet structure and function have been described as a consequence of malaria, and in rare instances platelets can be invaded by malarial parasites themselves.

A study conducted by John g kelton et al Immune-mediated Thrombocytopenia of Malaria from J. Clin. Invest. The American Society for Clinical Investigation, suggested that thrombocytopenia is a common finding in malaria, but the mechanism of the thrombocytopenia unknown. Initially it was suggested that DIC was responsible. Consistent with these observations are the studies between the amount of patient described in this report. Thrombocytopenic patients had elevated levels of PAIgG during the thrombocytopenic episode. The PAIgG returned to normal as the thrombocytopenia resolved, and while the patient continued on the same antimalaria drugs, indicating that the thrombocytopenia was not drug induced. [60]

Thrombopoietin (TPO) is the key growth factor for platelet production and is elevated in states of platelet depletion. TPO serum levels have been shown to be significantly higher in subjects with severe malaria normalizing within 14-21 days of therapy. Two types of changes in platelet dysfunction are seen in malaria. Initially there is platelet hyperactivity, this is followed by platelet hypoactivity. Platelet hyperactivity results from various aggregating agents like immune complexes, surface contact of platelet membrane to malarial red cells and damage to endothelial cells. The injured platelets undergo lysis intravascularly. The release of platelet contents can activate the coagulation cascade and contributes to DIC. Transient platelet hypoactivity is seen following this phase and returns to normal in 1 to 2 weeks [61,62].

In many studies undertaken, the significance of haemostatic abnormalities as a consequence of malaria has been difficult to assess as a result of the presence of various associated complications such as liver dysfunction, uraemia and treatment with low molecular weight dextran, dexamethasone and heparin. Absence of thrombocytopenia is uncommon in the laboratory diagnosis of malaria. Presence of thrombocytopenia is not a distinguishing feature between the two types of malaria. Thrombocytopenia less than 20,000/cmm can occur in P. vivax malaria although statistically more significant with P.falciparum malaria.

A case report by Kaur D et.al in 2007 on Unusual Presentation of Plasmodium vivax Malaria with Severe Thrombocytopenia and Acute Renal Failure was seen in 18 year old boy [63].

A study conducted by Kumar A et.al in 2006 on Thrombocytopenia-an indicator of acute vivax malaria suggested that thrombocytopenia as an early indicator for acute malaria; a finding that is frequent and present even before anemia and splenomegaly set in. The possible mechanisms leading to thrombocytopenia in malaria includes immune mechanisms, oxidative stress, alterations in splenic functions and a direct interaction between plasmodium and platelets [64]

A case report from the Department of Internal Medicine, Tokyo Medical College, found that the thrombocytopenia complicating some malarial infections is caused by immune mechanisms.A case of malaria associated with thrombocytopenia and increased platelet- associated IgG (PAIgG).In this case, anti-malarial therapy reduced the level of PAIgG to normal levels in association with normalization of the platelet count. This case suggests the immunological mechanisms of thrombocytopenia in malaria.[46]

Department of Internal Medicine, Fukaya Red Cross Hospital, Saitama, Japan, showed that severe thrombocytopenia in P.vivax malaria secondary to antibody mediated.[47]

A study conducted by Casals-Pascual C et.al in 2006 on Thrombocytopenia in falciparum malaria is associated with high concentrations of IL-10, suggested that platelets may play a role in the pathophysiology of severe malaria. However, somewhat paradoxically, thrombocytopenia is not associated clearly with outcome. When studied the relationship between thrombocytopenia and cytokines in Kenyan children with severe malaria, showed that thrombocytopenia (platelet count < 150 x 10(9)/L) strongly correlates with high levels of interleukin (IL)-10. Several studies have shown that high levels of IL-10 are associated with a favorable outcome in severe malaria. Taken together, these data suggest why thrombocytopenia has a complex relationship with severe disease and suggest one mechanism whereby IL-10 may modify the outcome of severe disease [65].

COAGULOPATHY:

Coagulation abnormalities are frequently found in patients with severe malaria. Clinically apparent bleeding or DIC is associated with very severe disease and high mortality rates. Bleeding in severe malaria

results from several pathological processes like thrombocytopenia, decreased clotting factor synthesis and consumptive coagulopathy. Activation of coagulation cascade occurs in even uncomplicated malaria but this is mild and reverts to normal as patients become afebrile and aparasitemic [66].

There is accelerated coagulation cascade activity with accelerated fibrinogen turnover, consumption of anti-thrombin 3, reduced factor 13 and increased concentration of fibrinogen degradation production in acute malaria32. Coagulation cascade is activated via intrinsic pathway [66]. In severe infection PT and PTT may be prolonged and in few patients (<5%) bleeding may be significant. Intravascular thrombus formation is observed rarely at autopsy in fatal cases. Fibrin deposition is sparse and platelets are strikingly unusual [67].

The high plasma levels of P-selectin found in severe malaria may be derived from platelets but could come from vascular endothelium, as plasma concentration of other endothelial derived proteins thrombomodulin, ICAM-1, VCAM-1 are elevated as well [68].

Sharma et al reported that incidence of DIC as 16.7% in their series and out of them only 3.3% had bleeding manifestations [20].

In a study conducted by Thomas Butler et al, out of 53 patients of acute falciparum malaria no one had classical DIC with bleeding. Abnormal coagulation tests sporadically observed were not accompanied by abnormalities with other tests that would establish DIC [69].

Renal dysfunction in falciparum malaria:

Renal failure in malaria is caused by renal cortical vasoconstriction and resultant hypoperfusion, sequestration and resultant acute tubular necrosis due to microvascular obstruction and due to massive intravascular hemolysis in blackwater fever. Dehydration and hypovolemia can lead to renal hypoperfusion, but this is reversible with adequate rehydration. High-grade fever, profuse sweating, lack of adequate intake, vomiting and diarrhea contribute to dehydration10,[70].

Acute Pulmonary Oedema:

Acute pulmonary oedema is a grave and usually fatal complication of severe falciparum malaria with more than 50% mortality. In a few patients it could be due to fluid overload as a result of over enthusiastic fluid therapy [71]. In others it develops even with normal or negative fluid balance. Pulmonary oedema develops later compared to other complications and it may even appear several days after treatment for malaria, when the patient is otherwise improving with a reduction in peripheral parasitemia.

The mechanism of pulmonary oedema is not clearly understood. It has a close resemblance to adult respiratory distress syndrome. While over-hydration may be the cause in some cases of pulmonary oedema, it can also develop in patients with normal capillary wedge pressures. Such cases may be due to increased permeability of pulmonary capillaries. Sequestration of red cells and clogging of pulmonary microcirculation and disseminated intravascular coagulation may also play their role. Pulmonary oedema is more common in patients with hyperparasitemia, renal failure and pregnancy and it is commonly associated with hypoglycemia and metabolic acidosis. It may develop suddenly after delivery, due to fluid overload40. Pulmonary oedema may be the terminal event in many cases of fatal falciparum infection. Increase in blood viscosity due to dehydration and hyperparasitemia also results in renal hypoperfusion. Intravascular hemolysis and clogging of the tubules by the products of hemolysis is another important cause for renal dysfunction. Severe falciparum malaria results in hemolysis of parasitized as well as non-parasitized red cells. Oxidant drugs like primaquine can also contribute to hemolysis in severe falciparum malaria, and particularly in patients with deficiency of Glucose 6-phosphate dehydrogenase enzyme. Although hemoglobin itself is not nephrotoxic, other products of hemolysis can cause acute tubular necrosis, particularly in the presence of dehydration and acidosis

Electrolyte imbalance:

Patients with severe falciparum malaria often have signs of dehydration (thirst, dry tongue, reduced ocular tension and reduced skin turgor) and hypovolumia (low central venous pressure, postural hypotension, oliguria with high urinary specific gravity). Mild hyponatremia (S. Sodium 125-135 mmol/L) is common.

Severe, symptomatic hyponatremia, however, is rare [72]. Metabolic acidosis may develop in severely ill patients with shock, hypoglycemia, hyperparasitemia or renal failure. Lactic acidosis is common in such patients and carries a high mortality [71]. Management of fluid balance is of utmost importance in severe falciparum malaria. While untreated dehydration and hypovolemia can result in hypoperfusion of kidneys, brain and other vital organs, thereby aggravating the complications, enthusiastic over- hydration can precipitate pulmonary oedema. Therefore, fluid balance should be managed carefully and meticulously [71].

Assess the status of hydration- moisture on the tongue, ocular tension, skin turgor, temperature of extremities, blood pressure and postural changes in blood pressure, peripheral venous filling and jugular venous pressure, urine output, urine specific gravity (>1.015 indicates dehydration), urinary sodium (<20 mmol/L indicates low renal perfusion).

CHRONIC MALARIA;

Patients with persistant vivax malaria may develop partial immunity with low grade parasitemia. They may develop progressive splenomegaly. The complication of chronic malaria is rupture of spleen, spontaneous or traumatic [73].

TROPICAL SPLENOMEGALY SYNDROME [74]:

Spectacular enlargement of the spleen in inhabitants of the tropics is almost always an expression of chronic malaria. The cardinal criteria for diagnosis of TSS are gross splenomegaly,hepatic sinusoidal infiltration, high titers of antibodies to malaria, high levels of IgM and responsiveness to antimalarials.Genetic factors undoubtedly play a role because within a malarious area the geographical distribution of TSS does not follow closely that of malarial transmission. The massively enlarged spleen leads to hypersplenism with anaemia, leucopenia and thrombocytopenia.

The hyperglobulinemia is believed to be resulting from polyclonal B-cell activation in the absence of adequate number of CD8+ suppressor T cells, which have been removed by an antibody dependent cytotoxic mechanism. This is due to excessive recticuloendothelial system stimulation due to production of

IgM antibodies to CD8 + T-cells, CD5 + T cells and increased CD4+/CD 8+ratio. These events may lead to uninhibited B-cell production of IgM and the formation of cryoglobulins. This causes reticuloendothelial hyperplasia and splenomegaly. Patients with tropical splenomegaly present with a dragging sensation in the abdomen and sometimes acute pain due to perisplenitis. Anemia and pancytopenia are present. Parasites are not found in peripheral blood. They are predisposed to respiratory and skin infections and can die of overwhelming sepsis. Refractory cases may evolve into lymphoproliferative disorders.The enlarged spleen usually regresses over a period of months with effective antimalarial prophylaxis.

The treatment of tropical splenomegaly syndrome involves administration of antimalarial prophylaxis for prolonged periods of time. This removes the antigenic stimulus provided by repeated malarial infection and allows the immune system to return to normal. The choice of antimalarial depends on the local sensitivity pattern. Chloroquine weekly or proguanil daily have been found to be useful.These drugs may have to be continued for long periods,possibly for life. Severe anemia may require blood transfusion Splenectomy is only recommended if there is an unequivocal failure of prophylaxis given for at least 6 months and there is severe hypersplenism [74] .

ATYPICAL PRESENTATION OF MALARIA:

Atypical features are more common in the following situations:

- Falciparum malaria
- Early infection
- Patients at extremes of age
- Patients who are immune-compromised (extremes of age, malnourished, AIDS, tuberculosis, cancers, on immunosuppressive therapy etc.)
- Patients on chemoprophylaxis for malaria
- Patients who have had recurrent attacks of malaria
- Patients with end stage organ failure
- Pregnancy.

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

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

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

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

Altered sensorium: Patients with P. falciparum malaria may present with altered sensorium due to severe infection, hypoglycemia, electrolyte imbalance due to vomiting or diarrhoea (particularly the elderly), hyperpyrexia, subclinical convulsions etc. Differential diagnosis will include acute encephalitis, meningitis, metabolic encephalopathy etc. As a rule of the thumb, malaria should be considered a possibility in all cases of acute neuropsychiatric syndromes and in cases of proven malaria, other possibilities should be considered in the presence of papilloedema, increasesd ICT, neck stiffness and focal deficits.

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

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

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

Chest pain: Acute retrosternal or precordial pain may be presenting feature of malaria. It may radiate to the left or right shoulder tips or arms. It is due to rapid increase in the splenic size and perisplenitis. This pain may mimic acute myocardial infarction, pleurisy, neuralgia etc. Coupled with breathlessness, sweating and hypotension (algid malaria), the picture will very closely resemble that of acute MI.

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

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

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

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

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

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

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

Hepatosplenomegaly: Patients can present with enlargement of liver and/or spleen, tender or non-tender, with or without fever. Rapid enlargement of spleen or liver in malaria can cause acute pain in the abdomen or chest. Generally, organomegaly is noticed in the second week of malarial illness. However, in cases of relapse or recrudescence, it may be present earlier. Also, in immune compromised patients spleenomegaly may be absent. In pregnancy, particularly second half, spleenomegaly may be smaller or an enlarged spleen

may regress in size due to immune suppression. Although spleenomegaly is a cardinal sign of malaria, absence of spleenomegaly does not rule out the possibility of malaria.

Malaria psychosis: Patients may present with altered behaviour, mood changes, hallucinosis or even acute psychosis, with or without fever. Malaria may be detected accidentally in such cases and they improve completely with anti malarial therapy. Altered behaviour may also be due to high grade fever or drugs. Antimalarial drugs like chloroquine, quinine, mefloquine and halofantrine can cause restlessness, hallucinations, confusion, delirium or even frank psychosis. In a study of 118 cases of malaria in Mangalore; Nagesh Pai, Satish Rao and B.S. Kakkilaya found varied psychiatric manifestations. Most of these patients were already on antimalarial treatment at the time of referral to the psychiatric service (unpublished data) [75].

DIAGNOSIS OF MALARIA :

The following laboratory procedures are available for diagnosis of malaria.

I. Microscopic

1. Light microscopy : Thick and thin blood smears
2. Fluorescent microscopy –
    a. Quantitative buffy coat
    b. Kawamoto acridine orange method
    c. Benzothiocarboxypurine procedure

II. Non Microscopic

3. Detecting specific nucleic – acid sequences: PCR based technique
4. Serological methods – Antigen detection
    a. HRP-2 based serological assays
    b. Parasite LDH (pLDH) based serological assays
    c. Plasmodium aldolase

5. Detection of Antimalarial Antibodies
6. Intraleucocytic malaria pigment
7. Flowcytometry
8. The Rapid Malaria Tests

A. Light Microscopy : Thick and thin blood smears The accepted laboratory procedure and the gold standard for the diagnosis of malaria is the preparation and examination of Giemsa or Field – stained blood smear under the microscope. Blood obtained by pricking a finger is the ideal sample but blood obtained by venipuncture and collected in heparin or EDTA coated tubes is acceptable if used shortly after it is drawn. Both thick and thin smears should be made.

Thin smear: should be rapidly air-dried, fixed in anhydrous methanol, and stained and RBC's in the tail of the smear should be examined under oil immersion (1000 magnification). The level of parasitemia is expressed as number of parasitized erythrocytes per 1000 RBC's, or per 200 WBC's, and this figure is converted to number of parasitized erythrocytes per microliter. Count the number of parasites within 1000 red blood cells and divide this by 10. This gives the percentage of parasitemia. A parasite count of 100000 or more per mm3 (or 5% and more) is considered as severe infection.

Thick smear: should be of uneven thickness, and it should be dried thoroughly, and stained without fixing. As many layers of RBC's overlie one another and are lysed during the staining, the thick film has the advantage of concentrating the parasites (by 20-40 fold) and thus increasing the diagnostic sensitivity. Both parasites and WBC's are counted and the number of parasites per unit volume is calculated from the total leukocyte count. Count the number of asexual forms of the parasite (rings, trophozoites and schizonts) against 100 leukocytes and multiply by 75, this gives an approximate total per micro liter (mm3). The average leukocyte count per microscopic field is about ten. Therefore, multiply the average number of parasites per field by 750, this also gives an approximate total per micro liter. The thick smear, which concentrates by a factor of 20-30 layers of RBC on a small surface, provides the sensitivity of the technique and is much better than the thin smear for detection of

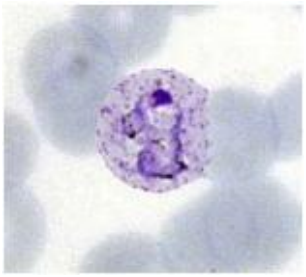
malarial infection. The thin smear gives the test its specificity, being much better than the thick smear for species identification and evaluation of intensity of parasitaemia.

Parasite count: This is a simple yet very important and useful method of assessing the severity of infection in falciparum malaria. It should be done routinely in all cases of falciparum malaria. An experienced technician can detect as few as 5 parasites/ l in a thick film and 200/ l in a thin film.

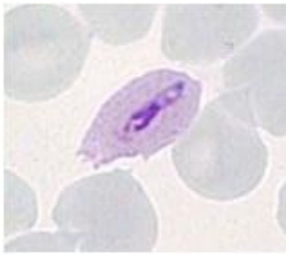
Staining methods: 1. Giemsa 2. Lieshman's 3. Jaswanth Singh Battacharya 4. Wright's 5.Field's. Among these Giemsa's stain is preferred.

Fig. 4 : Peripheral smear in malaria

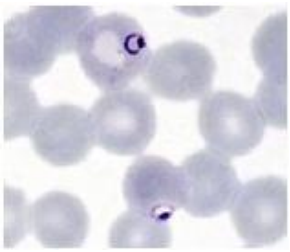
Trophozoites :



P.vivax

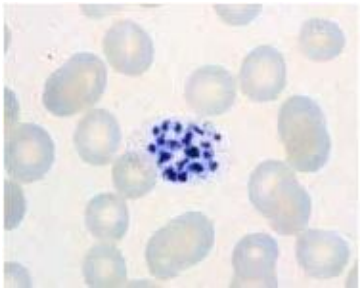


P. ovale

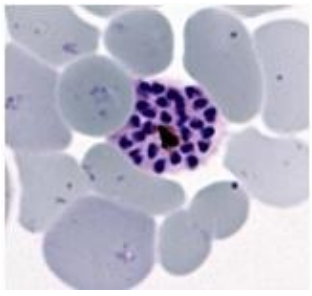


P.falciparum

Schizonts :

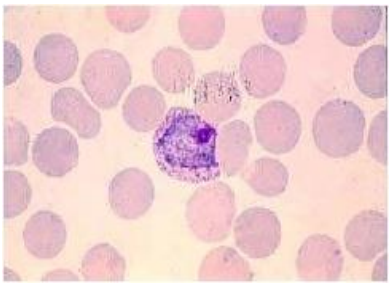


P. vivax

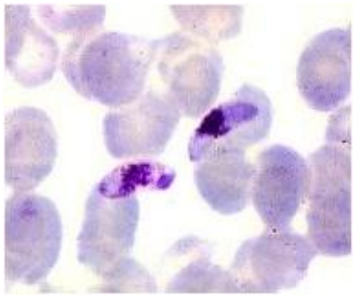


P. falciparum

Gametocytes:



P. vivax



P.falciparum

Disadvantages of peripheral smear examination :

1. It takes upto 60 minutes of preparation time.[9]
2. It is labour intensive.
3. Interpretation of results requires considerable expertise particularly at low levels of parasitaemia.
4. Can miss falciparum malaria as the parasites can be sequestered and are not always present in peripheral blood.[10]
5. Does not differentiate between dead and live parasites.

Advantages :

1. Cheap.
2. Can quantify the parasite load (thick smear).

a. One method is to count the total number of parasites per 200 WBC and multiply this number by 40 to give the number of parasites / ml after assuming that there are always 8000 WBC / ml of blood.

b. Second method involves making a thick smear with a known small volume of blood (0.3ml) and then counting all the parasites on the smear. The total parasite count is multiplied then by 3.33 to obtain the number of parasites / ml.

A study conducted by Kakkilaya BS. Rapid Diagnosis of Malaria. Lab Medicine. 2003 Aug;[76] concluded although the peripheral blood smear examination that provides the most comprehensive information on a single test format has been the "gold standard" for the diagnosis of malaria, the immunochromatographic tests for the detection of malaria antigens, developed in the past decade, have opened a new and exciting avenue in malaria diagnosis. However, their role in the management and control of malaria appears to be limited at present [77].

B. Fluorescent microscopy : The three techniques currently available are rapid and relatively easy to perform (when there are >100 parasites /ml) and demonstrate sensitivities and specificities equivalent to those achievable by examination of stained thick smears.

Both the QBC and Kawamoto methods use acridine orange as the fluorochrome. The specificity of acridine orange staining for P.vivax is 52% where as for P.falciparum it is around 93%.11 The sensitivity at <100 parasites / ml is between 41.7% - 93%. The BCP method has a reported sensitivity and specificity of > 90%.

An important limitation of method based on AO and BCP is their inability to differentiate between plasmodium subspecies.

a) Quantitative Buffy Coat (QBC) Test

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.

Method: The QBC tube is a high-precision glass hematocrit tube, pre-coated internallywith 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.

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.

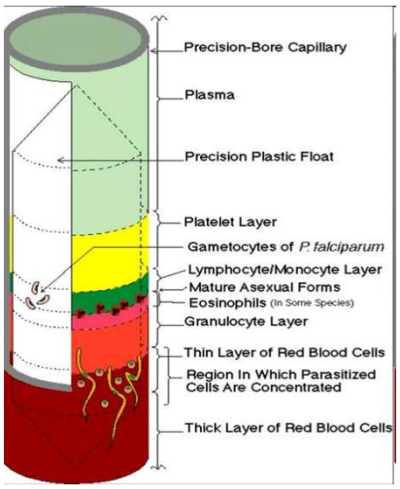
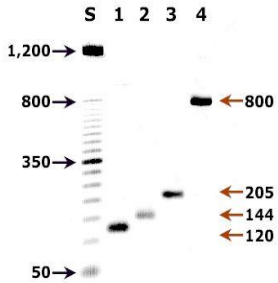


Table 2: Comparision of peripheral smear and QBC

	Peripheral smear	QBC
Method	Cumbersome	Easy
Time	Longer, 60 - 120 minutes	Faster, 15 - 30 minutes
Sensitivity	5 parasites/ l in thick film and 200 / l 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

Polymerase Chain Reaction (PCR) :

The major advantage of using a PCR based technique is the ability to detect infection in patients with low parasitaemias; infections with 5 parasites/ml can be detected with 100% specificity.[78] However such techniques are expensive and labour intensive, require extensive technical expertise and cannot distinguish between viable and non-viable organisms.



A: Agarose gel (2%) analysis of a PCR diagnostic test for species-specific detection of *Plasmodium* DNA. PCR was performed using nested primers of Snounou et al.[1]

- **Lane S:** Molecular base pair standard (50-bp ladder). Black arrows show the size of standard bands.
- **Lane 1:** The red arrow shows the diagnostic band for *P. vivax* (size: 120 bp)
- **Lane 2:** The red arrow shows the diagnostic band for *P. malariae* (size: 144 bp)
- **Lane 3:** The red arrow shows the diagnostic band for *P. falciparum* (size: 205 bp)
- **Lane 4:** The red arrow shows the diagnostic band for *P. ovale* (size: 800 bp)

C. Serological methods : Antigen detection :

D. The new generation, antigen capture tests are capable of detecting fewer parasites and produce rapid results (10-15 min). They are commercially available as kits, which include all the necessary reagents and do not require extensive training or equipment to perform or to interpret their results.

Two antigens are currently available for detection :

a) Histidine rich protein-2 (HRP-2) which is only produced by P. falciparum.[79,80]

b) Parasite lactate dehydrogenase (pLDH) produced by all 4 species infecting man.

c) Plasmodium aldolase

Both these antigens are secreted into the blood by all sexual sages of the parasite. The pLDH is also produced by gametocyte.[81] The latest antigen capture tests are rapid simple to perform and have detection limits comparable to those of high quality microscopy.

a) HRP –2 based serological assays :

Histidine-rich protein 2 of P. falciparum (PfHRP2) is a water soluble protein that is produced by the asexual stages and gametocytes of P. falciparum, expressed on the red cell membrane surface, and shown to remain in the blood for at least 28 days after the initiation of antimalarial therapy. Several RDTs targeting PfHRP2 have been developed41. When there are > 60-100 parasites / ml, the HRP-2 based tests are >90% sensitive and >90% specific compared to thick smear microscopy. Currently two such tests are available. The parasite F test and the ICT Malaria Pf test. These detect only P. falciparum malaria and are based on monoclonal antibodies to HRP-2 which are immobilized in nitrocellulose strips.

Disadvantages : Detects only P. falciparum.

HRP - 2 persists in the blood long after the clinical symptoms of malaria have disappeared and the parasites have apparently been cleared from the host.

b) p-LDH based serological assays :

Parasite lactate dehydrogenase (pLDH) is a soluble glycolytic enzyme produced by the asexual and sexual stages of the live parasites and it is present in and released from the parasite infected erythrocytes. It has been found in all 4 human malaria species, and different isomers of pLDH for each of the 4 species exist. With pLDH as the target, a quantitative immunocapture assay, a qualitative immunochromatographic dipstick assay using monoclonal antibodies, an immunodot assay, and a dipstick assay using polyclonal antibodies have been developed41. These tests are sensitive, specific and easy to perform with results obtainable in < 15 min. The pLDH based assays also differentiate between P. falciparum and other plasmodium subspecies and since pLDH is only produced by viable parasites, they are also useful in monitoring anti-malarial therapy.

The pLDH – based assays are available in two formats : a semiquanitative, dry, dipstick (OPTIMAL) and a 96-well, quantitative, immunoenzymatic capture assay.[81] The optimal assay is a versatile test, having the ability to both diagnose malaria and follow during therapy. In addition, this test can be used as an epidemiological tool since, in areas where more than one species of plasmodium is circulating, it can be used to identify the plasmodium species infecting the patients in each village quickly and allow the public – health workers to deliver the most appropriate chemotherapy.

c) Plasmodium aldolase

This is an enzyme of the parasite glycolytic pathway expressed by the blood stages of P. falciparum as well as the non-fa1ciparum malaria parasites.Monoclonal antibodies against Plasmodium aldolase are pan-specific in their reaction and have been used in a combined 'P.f/P.v' immunochromatographic test that targets the pan malarial antigen (PMA) along with PfHRP241.

Detection of Antimalarial Antibodies:

Antibodies to the asexual blood stages appear a few days after malarial infection, increase in titre over the next few weeks, and persist for months or years in semi-immune patients in endemic areas, where re-infection is frequent. In non-immune patients, antibodies fall more rapidly after treatment for a single infection and are undetectable in 3-6 months. Re-infection/relapse induces a secondary response with a rapidly increasing antibody titer [82]. Malarial antibodies can be detected by immunofluorescence or enzyme immuno assay. It is useful in epidemiological surveys, for screening potential blood donors and occasionally for providing evidence of recent infection in non-immunes. In future, detection of protective antibodies will be important in assessing the response to malaria vaccines.

Intraleucocytic malaria pigment:

Intraleucocytic malaria pigment has been suggested as a measure of disease severity in malaria. In a study of 146 children aged 6 months to 14 years in 4 categories - cerebral malaria, mild malaria, asymptomatic malaria and 'no malaria'- in Ibadan, Nigeria, an area of intense malaria transmission in Africa, the proportion of pigment-containing neutrophils showed a clear rise across the spectrum no malaria--asymptomatic malaria--mild malaria--cerebral malaria (median values 2.0%, 6.5%, 9.0% and 27.0%, respectively; $P < 0.0001$). The proportion of pigment-containing monocytes did not differ significantly between the mild malaria, asymptomatic malaria and no malaria groups but the cerebral malaria group had a higher median value than the other 3 groups. The ratio of pigment-containing neutrophils to pigment-containing monocytes showed the same trend across the groups of subjects as was observed with the number of pigment-containing neutrophils. The study concluded that the pigment- containing neutrophil count is a simple marker of disease severity in childhood malaria in addition to the parasite count [83].

Flow cytometry:

Flow cytometry and automated hematology analyzers have been found to be useful in indicating a diagnosis of malaria during routine blood counts. In cases of malaria, abnormal cell clusters and small particles with DNA fluorescence, probably free malarial parasites, have been seen on automated hematology analyzers and it is suggested that malaria can be suspected based on the scatter plots produced

on the analyzer. Automated detection of malaria pigment in white blood cells may also suggest a possibility of malaria with a sensitivity of 95% and specificity of 88%. On flow cytometric depolarized side scatter, the average relative frequency of pigment carrying monocytes was found to differ among semi-immune, non-immune and malaria negative patients [82].

The Rapid Malaria Tests:

The RDTs have been developed in different test formats like the dipstick, strip, card, pad, well, or cassette; and the latter has provided a more satisfactory device for safety and manipulation. The test procedure varies between the test kits. In general, the blood specimen (2 to 50µL) is a finger-prick blood specimen, anticoagulated blood, or plasma, and it is mixed with a buffer solution that contains a hemolyzing compound and a specific antibody that is labelled with a visually detectable marker such as colloidal gold. In some kits, labelled antibody is pre-deposited during manufacture and only a lysing/washing buffer is added. If the target antigen is present in the blood, a labelled antigen/antibody complex is formed and it migrates up the test strip to be captured by the pre-deposited capture antibodies specific against the antigens and against the labelled antibody (as a procedural control). A washing buffer is then added to remove the hemoglobin and permit visualization of any colored lines formed by the immobilized antigen-antibody complexes. The pLDH test is formatted to detect a parasitemia of >100 to 200 parasites/µL and some of the PfHRP2 tests are said to detect asexual parasitemia of >40 parasites/µL [82].

Sensitivity: RDTs for the diagnosis of P. falciparum malaria generally achieve a sensitivity of >90% at densities above 100 parasites per L blood and the sensitivity decreases markedly below that level of parasite density. Many studies have achieved >95% sensitivity at parasitemia of ~500 parasites/ L, but this high parasitemia is seen in only a minority of patients. For the diagnosis of. P vivax malaria, the PfHRP2/PMA test has a lower sensitivity compared to that for P. falciparum malaria; however, the pLDH test has an equal or better sensitivity for P. vivax malaria compared to P. falciparum malaria. For the diagnosis of P. malariae and P. ovale infections, the sensitivity is lower than that of P. falciparum malaria at all levels of parasitemia on both the PfHRP2/PMA and the pLDH tests. The specificity appears to be better with the pLDH test than the PfHRP2/PMA test for both P. falciparum and non-falciparum malaria.

False Positivity: False positive tests can occur with RDTs for many reasons. Potential causes for PfHRP2 positivity, other than gametocytemia, include persistent viable asexual-stage parasitemia below the detection limit of microscopy (possibly due to drug resistance), persistence of antigens due to sequestration and incomplete treatment, delayed clearance of circulating antigen (free or in antigen-antibody complexes) and cross reaction with non-falciparum malaria or rheumatoid factor. Proportion of persistent positivity has been linked to the sensitivity of the test, type of test, degree of parasitemia and possibly the type of capture antibody.

False negativity: On the other hand, false negative tests have been observed even in severe malaria with parasitemias >40,000 parasites/ l. This has been attributed to possible genetic heterogeneity of PfHRP2 expression, deletion of HRP-2 gene, presence of blocking antibodies for PfHRP2 antigen or immune-complex formation, prozone phenomenon at high antigenemia or to unknown causes Cross reactions between Plasmodia species and problems in identifying non- falciparum species: Cross reaction of PfHRP2 with non-falciparum malaria could give false positive results for P. falciparum and mixed infections containing asexual stages of P. falciparum could be interpreted as negative in about one third of the patients.

Another major difficulty still encountered by the use of RDTs is the correct identification of Plasmodium species, particularly in areas where nonfalciparum malaria is prevalent. The PfHRP2 tests can detect only P. falciparum infection and would miss the more common non-falciparum malaria in areas where other Plasmodium species are co-endemic.

TREATMENT OF MALARIA :

The aim of chemotherapy in malaria is both complete clinical cure of the individual patient and prevention of spread of the parasite from patient to others. Apart from the specific antimalarial drug treatment, treatment of complications is also important.

Anti malarial drugs can be classified according structure

1. Aryl amino alcohols: Quinine, quinidine (cinchona alkaloids), mefloquine, halofantrine.
2. 4-aminoquinolines: Chloroquine, amodiaquine.
3. Folate synthesis inhibitors:

    a. Type 1 - competitive inhibitors of dihydropteroate synthase - sulphones, sulphonamides
    b. Type 2 - inhibit dihydrofolate reductase - biguanides like proguanil and chloroproguanil; diaminopyrimidine like pyrimethamine

4. 8-aminoquinolines:Primaquine, WR238, 605
5. Antimicrobials: Tetracycline, doxycycline, clindamycin, azithromycin, fluoroquinolones
6. Peroxides: Artemisinin (Qinghaosu) derivatives and analogues - artemether, arteether, artesunate, artelinic acid
7. Naphthoquinones:Atovaquone
8. Iron chelating agents:Desferrioxamine

1. CHLOROQUINE : It is a 4- aminoquinoline which has marked and rapid schizonticidal activity against infections of all species of the parasite. It also kills gametocytes of P. vivax.

The dose is 25mg/kg body weight given over three days. When used for chemoprophylaxis 5 mg/kg weekly is the dose. No abortifacient or teratogenic effect has been noted with the use of chloroquine. Therapeutic concentration in plasma is reached within 30 min. It has a elimination half life of 10 days. Transient nausea and gastrointestinal symptoms may be seen. Pruritus may be seen in dark skinned people. Temporarily blurred vision may be seen. Choloroquine has a low margin of safety especially in children. Acute poisoning is very dangerous. Death may result with a single dose of 1.5-2 gram even in adults. Symptoms may include headache, nausea, diarrhoea, dizziness, muscular weakness and blurred vision which may progress dramatically to loss of vision. Cardiovascular toxicity with hypotension and arrhythmmias may be seen progressing on to collapse.Resistance to chloroquine has been noted in many countries. The following WHO guidelines assist in grading the resistance to 4- inoquinolones16 -

Sensitive - clearance of parasitemia within 7 days of initiation of therapy without subsequent recrudescence.

2. Low grade resistance - clearance of parasitaemia followed by recrudescence.

3. High grade resistance – greater than 75% but less than 100% parasites cleared within 7 days.

4. High grade resistance – parasite count does not fall by more than 75%.

2. PRIMAQUINE : It is an 8-aminoquinoline highly active against gametocytes of all human malaria species and hypnozoites of P. vivax. Primaquine is readily absorbed when taken orally. It has a plasma half life of about 5 hours. The adverse effects include haemolysis especially in those with G-6 PD deficiency. Similarly haemolysis occurs in RBC's with fetal haemoglobin, hence should not be used in infants and in pregnant females. Primaquine may cause methaemoglobinemia or suppression of myeloid activity. Folinic acid may be used as an antidote.
3. QUININE : It is the drug of choice in severe and complicated cases of P. falciparum. It can cause severe hypotension if injected rapidly because of its cardiovascular suppressant activity. It also causes hypoglycemia as it stimulates insulin release from pancreatic □-cells. It is safe in pregnancy. In uncomplicated malaria, it should be reserved for cases of chloroquine resistance.
4. QUINIDINE : Although superior to quinine in its anti-malarial activity, its main drawbacks are increased cost and lethal side effects like cardiac arrhythmmias and hypersensitivity.
5. SULFA DRUG PYRIMETHAMINE COMBINATION: They are highly active against schizonts of P. falciparum but less active against P. vivax. They have no activity against gametocytes. They should be used against chloroquine resistant P. falciparum infection which is not complicated. They are contraindicated in pregnancy and lactation and in young children. Adverse effects include hypersensitivity involving skin and mucus membrane, Steven Johnson syndrome, megaloblastic anaemia and pancytopenia.

6. AMODIAQUINE : It is used in chloroquine failure as a primary drug and is more effective in clearing parasitaemia. Side effects like pruritus, toxic hepatitis and fatal agranulocytosis prevent its widespread use.
7. MEFLOQUINE : It was the first synthetic quinoline methanol compound to be introduced as an antimalarial drug and its sensitivity is independent of resistance to 4-aminoquinoline and diyhydrofolate reductase inhibitors. It is a blood schizonticidal drug with high affinity for erythrocyte membranes where it preferentially binds with phospholipids. One great advantage is that it can be given as a single dose. The drug is not recommended in children below 5 kg and less than 3 months of age. The toxic side effects include dizziness, nausea, vomiting, arrhythmmias and acute brain syndrome consisting of fatigue, asthenia, seizures and psychosis. It should be avoided in patients with known hypersensitivity, patients on beta-blockers, pregnancy, epileptic and psychiatric patients.

Drug	Primary Tissue phase	Erythrocytic phase		Hypnozoites	Sporozoites
		Asexual phase	Gametocyte		
Quinine	No action	Fast action	Against vivax	No action	No action
Chloroquine	No action	Fast action	Against vivax	No action	No action
Primaquine	Active	Active only in toxic dose	Direct and fast action	Highly active	Highly active
Sulfa-pyri-methamine	Little action on falciparum	Slow and incomplete less on vivax	No action	No action	Less action
Mefloquine	No evidence	Active especially on P. falciparum	No evidence	No evidence	No evidence

8. Table 3 : Action of common antimalarials.8

HALOFANTRINE : [84]

It is a phenanthrene – methanol effective against multidrug resistant strains. It is a schizonticide for all 4 malarial species and acts by concentrating and combining with ferriprotoporphyrin IX in the parasite to form toxic complexes that damage biomembranes. It has only mild GI disturbances as side effects. Treatment with halofantrine in patients in whom mefloquine treatment or prophylaxis has failed may be less effective due to cross resistance between halofantrine and mefloquine. [85]

QUINHAOSU (Artemesnin and its derivatives)

Also called sweet wormwood, it has been used in traditional Chinese medicine for atleast 2000 years. It is an endoperoxide of a sesquiterpenoid lactone. The various derivatives are :-

Artemesnin

Dihydroartemesnin

Artemether

Artesunate

Arteether

Artesunate is an exciting development in the treatment of multi drug resistant malaria. According to WHO guidelines18 artesunate should be used only for the treatment of uncomplcated P.falciparum malaria in areas where resistance to mefloquine and or quinine has been demonstrated. The safety of artemesnin and its derivatives has not been established during the first trimester of pregnancy. Use of higher doses 600-1200 mg may cause dizziness, itching, abdominal pain, diarrhoea, vomiting and hair loss.

10.TETRACYCLINES AND CLINDAMYCIN : These drugs have a place in the treatment of drug resistant malaria when used in combination with quinine by enhancing the efficiency of treatment.

11.ANTIFUNGALS :

Ketoconazole, itraconazole, and amphotericin B have been found to be effective invitro for chloroquine resistant P. falciparum infection.

12. OTHER NEWER DRUGS / EXPERIMENTAL DRUGS.

1. WR – 33063
2. Cysteine and aspartic proteinase inhibitors
3. Pyronaridine 19
4. Azithromycin
5. Atovaquone

13. MISCELLANEOUS DRUGS

Benflumenthol

Hydroxypiperaquine

Trioxanes, tetraoxanes, peroxides

Hydroxynaphthquinones

Pyridinano- methanols.

Lead compounds.

Desferoxamine

Most of these compounds may be useful in drug resistant malaria, although further evaluation is required .

TREATMENT REGIMENS [86]

Treatment of infection with all species (except chloroquine resistant Pl.falciparum or pl.vivax).

Oral treatment of uncomplicated Pl.falciparum or pl. malariae infection: Chloroquine phosphate 1 gm as initial dose, then 0.5gm at 6, 24 and 24 hrs.

Oral treatment of pl.vivax, pl. ovale or species unidentified: Chloroquine phosphate 1gm as initial dose, then 0.5gm at 6, 24 and 48 hrs and on days 10 and 17 plus primaquine 52.6mg daily for 14 days.

Treatment of severe attacks: Parenteral quinine dihydrochloride or quinidine gluconate. Start oral chloroquine therapy as soon as possible; follow with primaquine if needed. Or Parenteral artesunate, artemether or chloroquine until the patient can take oral chloroquine, follow with primaquine if needed.

Treatment of infection with chloroquine resistant Pl.falciparum or pl.vivax

- Oral treatment of uncomplicated of Pl. falciparum resistant to chloroquine: Malarone two tablets twice daily with food for 3 days (each tablet contains atovaquone250 mg and proguanil100mg). or
- Quinine sulphate 10mg/kg 3 times daily for 3-7 days, plus one of the following-

  1. Doxycycline 100mg twice daily for 7 days.

  2. Clindamycin 7mg/kg 3 times daily for 7 days. 3. Tetracycline 250-500mg 4 times daily for 7 days

  3. Tetracycline 250-500mg 4 times daily for 7 days

1. Artesunate 4mg/kg/day for 3 days, plus Mefloquine(750 mg followed by 500 mg 12 hours later)
2. Mefloquine 750 mg followed by 500 mg 6-12 hours later.
3. Atovaquone/doxycycline, 500mg/100mg twice daily for 3 days.

0ral treatment of pl.vivax resistant to chloroquine.

- Malarone two tablets twice daily with food for 3 days (each tablet contains atovaquone 250 mg and proguanil100mg).or
- Mefloquine 750 mg followed by 500 mg 6-12 hours later.
- Quinine sulphate 10mg/kg 3 times daily for 3-7 days, plus Doxycycline 100mg twice daily for 7 days.

Parenteral treatment of severe attacks;

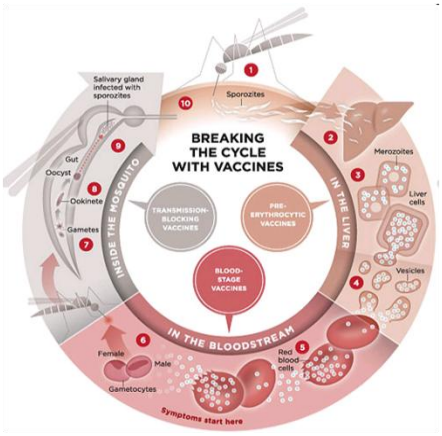
- Artemether intramuscularly (3.2mg/kg on day 1 followed by 1.6mg/kg daily for 3 days) , plus oral Mefloquine(750 mg followed by 500 mg 12 hours later)
- Artesunate intravenous(2.4mg/kg on day one followed by 1.2 mg/kg for 3 days) , plus oral Mefloquine(750 mg followed by 500 mg 12 hours later)
- Quinine dihydrochloride or quinidine gluconate plus intravenous doxycycline, tetracycline or clindamycin. Start oral therapy as soon as possible to complete the course of treatment.

Treatment of haematological complications [87]:

Treatment of haematological complications is symptomatic. If the patients Hb is less than 7gm% then blood transfusion is advised, preferably packed cells. If the patient has platelet count less than 50,000 per cumm, then platelets transfusion was advised.

MALARIA VACCINE [88]

So many efforts have been directed to develop multi-stage, multi-component vaccine, incorporating multi-antigenic sequences from different asexual and sexual stages of plasmodia. About nine different malaria antigens have been identified, which may not be the end of the road. The Indo-US researchers have combined the coding sequences for these key portions, called epitopes into one synthetic gene as the basis for the new vaccine named CDC\NII Malvac-1. The monkey trials will be followed by human trials.



Classification of Malaria Vaccines43

Stage of plasmodium	Antigen	Sensitivity
Pre-erythrocytic	Irradiated sporozoites, Circum Sporozoite Protein (CSP) or peptides, Liver stage Antigens -1 (LSA-1)	Stage/species specific; antibody blocks infection of liver; large immunizing dose required; can abort an infection
Merozoite and Erythrocytes	Erythrocyte Binding Antigen (EBA-175), Merozoite Surface Antigen 1&2 (MSA-1&2); Ring Infected Erythrocyte Surface Antigen (RESA); Serine Repeat Antigen (SERA); Rhoptry Associated Protein (RAP); Histidine Rich Protein (HRP); Apical Membrane Antigen-1 (APM-1)	Specific for species and stage; Cannot abort an infection; Prevents invasion of erythrocytes, thus reducing severity of infection

Gametocytes & gametes	Pfs 25, 48/45k, Pfs 230	Prevents infection of mosquitoes; antibody to this antigen prevents either fertilization or maturation of gametocytes, zygotes or ookinetes; is of use in endemic areas but not suited for travellers; antibody blocks transmission cycle
Combined vaccine (cocktail)	SPf 66 (based on pre-erythrocytic and asexual blood stage proteins of Pf)	Based on incorporation of antigens from different stages into one vaccine to produce an immune response, blocking all stages of the parasite development

Table No. 6 Examples of malarial vaccines [88]:

C.S.P. Vaccine: Kenyan study concluded that CSP vaccine induced antisporozoite antibody is not protective. Encouraging results have been reported with a CSP-HBs Ag Hybrid Vaccine (U.S. Army and SKB).

NYVAC - Pf. 7: This vaccine blocks transmission of the parasite from vertebrate host to mosquitoes. The highly attenuated NYVAC vaccinia virus strain has been utilized to develop a multiantigen, multistage vaccine candidate for malaria. Genes encoding seven Plasmodium falciparum antigens derived from the sporozoite (circumsporozoite protein and sporozoite surface protein 2), liver (liver stage antigen 1), blood (merozoite surface protein 1, serine repeat antigen, and apical membrane antigen 1), and sexual (25-kDa sexual-stage antigen) stages of the parasite life cycle were inserted into a single NYVAC genome to generate NYVAC-Pf7. Each of the seven antigens was expressed in NYVAC- Pf7-infected culture cells, and the genotypic and phenotypic stability of the recombinant virus was demonstrated. When innoculated into rhesus monkeys, NYVAC-Pf7 was safe and well tolerated. Antibodies that recognize sporozoites,

liver, blood, and sexual stages of P. falciparum were elicited. Specific antibody responses against four of the P.falciparum antigens (circumsporozoite protein, sporozoite surface protein 2, merozoite surface protein 1, and 25-kDa sexual-stage antigen) were characterized. The results demonstrate that NYVAC-Pf7 is an appropriate candidate vaccine for further evaluation in human clinical trials.

Recombinant Vaccine: Against P. vivax blood stage infection, a recombinant C- terminal fragment of MSP-1 in block co-polymer adjuvant with T- helper epitopes, the yeast expressed P2 P30 PV20019 recombinant vaccine offers partial protection in Saimiri monkeys. Combination of malarial antigens with immune boosting adjuvants and hepatitis B surface antigens has been reported. Liver stage vaccine may hold the key to reduce relapse/ re-infections in malaria prone individuals [88].

Gamete Vaccine: When the antibodies are taken up by the mosquitoes, gametes escaping the RBCs will be neutralised, thus preventing fertilisation and reducing transmission.

NA Vaccine: Based on a synthetic gene, made by adding 21 epitopes of 9 different antigens present in Pl. falciparum. Epitopes are small regions in proteins, which are recognized by immune cells. This has been developed by CDC with National Institute of Immunology. Example - Pf 155/RESA (Ring Infected Erythrocyte Surface Antigen) Naked DNA vaccine is capable of developing 'killer' cytotoxic T Lymphocyte (CTL) responses which are dose related. Stimulation of an immune response through the introduction of foreign genes resulting in the production of foreign genes and foreign protein is the basis of DNA vaccine [88].

CDC/NII MLVAC-1: It is a candidate vaccine that codes for nine different antigens that the plasmodium expresses during its development is liver, blood and circulation in the hosts. The vaccine stood the challenge on rabbit trial.

Patorraya Vaccine (Cocktail vaccine): Although knowledge of the parasite's biology is incomplete, research has allowed insight into some of the mechanisms that the parasite uses to evade host immunity. This is the basis for adopting an "antigenic cocktail" approach toward obtaining a synthetic or recombinant subunit vaccine such as the synthetic Colombian Malaria vaccine SPf 66. SPf 66 consists of 3 peptide

epitopes from 3 blood stage proteins (35 KD, 55KD,83 KD) intercalated with NANP sequence. This vaccine is designed to block the parasite at its later merozoite form, when it emerges from initial incubation in liver. The vaccine stimulates production of antibodies, which would prevent the parasite from infecting RBCs. During the development of Spf 66, field trials under both low and high malaria endemicity areas in Latin America and Africa have been carried out, at a dosage of 1 mg for children < 5 years and 2 mg for adults over deltoid on days zero, 30 and 180 days. The results from these studies showed a protective efficacy ranging between 38.8 and 60.2% against Plasmodium falciparum malaria. In Tanzania, the efficacy has been 31% in children (1-5 yrs old), while protective efficacy in Gambia was 8% (in infants 6-11months old).SPf 66 with QS - 21 adjuvant is also undergoing trials [88].

Conclusion: Sporozoite vaccines containing CSP, generated by recombinant DNA technology, combined with potent T-cell epitopes for higher immunogenecity seem to inhibit early liver-stages of the parasite and may be compared to causal prophylaxis.

Blood stage antigens combined with Freund's adjuvant or other immunoboosters may generate effects resembling chloroquine chemoprophylaxis. Merozoite vaccines get longer time to interact with their target than the transiently appearing sporozoites in case of Sporozoite vaccines. Gamete vaccines hold promise for future where in antibodies when taken up by the mosquitoes will neutralise the gametes escaping RBC's, thus preventing fertilization. This will reduce transmission and a reduction in nosocomial infection. The cocktail vaccine is the most promising one [88].

MATERIALS AND METHODS

SOURCE OF DATA

All patients above 18 years with fever and proved to be having acute malaria admitted to B.L.D.E.U's Shri B.M.Patil Medical College, Hospital and Research Center Bijapur, between November 2009 to April 2011

INCLUSION CRITERIA

a) All patients above 18 years with history of fever and peripheral smear study, QBC test or antigen test positive for malarial parasite.

EXCLUSION CRITERIA

a) Cases who have undergone treatment before giving a blood sample

b) History of - Congenital & Hereditary Thrombocytopenia

Immune induced thrombocytopenia

Drug induced thrombocytopenia.

c) Only gametocyte is seen in peripheral smear and patient has no history of fever.

SAMPLE SIZE : 70 cases of malaria as diagnosed by peripheral smear examination.

STATISTICAL ANALYSIS

Statistical Methods:

a) Proper statistical tests i.e. chi square test

fischer exact test

student 't' test

b) Diagrammatic representation

Chi square test or Fisher Exact test and student 'T' test has been used to find the significant association of study characteristics (Thrombocytopenia) with type of malaria.

1. Chi-Square Test

$$\chi^2 = \sum \frac{(Oi - Ei)^2}{Ei}$$

Where Oi is observed frequency and Ei is Expected frequency

2. Fisher Exact Test

$$\text{Fisher Exact Test statistic} = \sum p = \frac{(a+b)!\,(c+d)!\,(a+c)!\,(b+d)!}{n!} \quad \frac{1}{\sum a!b!c!d!}$$

4. Classification of Effect size

No effect	d<0.20
Mild effect	0.20 <d<0.50
Moderate effect	0.50 <d<0.80
Large effect	0.80<d<1.20
Very large effect	d>1.20

Study design

A total of 70 patients diagnosed to have Malaria over a period of two years admitted in BLDEU Shri B.M. Patil Medical College and Hospital included in the study. This is a prospective study. All study subjects were identified positive for Malaria parasite on peripheral smear examination with conventional microscopy. Platelet count was done on a fully automated, quantitative analyzer. Platelet count was the number of thrombocytes derived from directly measured platelet pulses, multiplied by a caliberation constant and expressed in thousands of thrombocytes per microliter of whole blood. Repeat platelet count

were done in subjects with marked thrombocytopenia until normal or near normal values were reached. P.falciparum antigen test (PfHrp antigen test-Parascreen) was performed in all subjects with malaria parasite positive on peripheral smear.P.vivax Malaria on the peripheral smear with a platelet count less than 20,000cells/cmm for more emphatic exclusion of associated P.falciparum infestation. P.falciparum antigen test was also performed in subjects with high index of clinical suspicion or multi organ involvement. Other investigation includes CBC, LFT, RFT, Chest X- Ray, Ultrasound Abdomen, if necessary Blood Culture, Urine Culture, Dengue and Leptospiral serology. P.falciparum was treated with either chloroquine or artesunate depending upon the clinical severity. P.vivax malaria was treated with chloroquine followed by two weeks course of primaquine. Data was expressed on a excel spreadsheet and statistical analysis was performed. P values less than 0.005 were considered significant.

	Class1	Class2	Total
Sample1	a	b	a+b
Sample2	c	d	c+d
Total	a+c	b+d	n

Method of collection of data :

Information was collected through prepared proforma for each patient.

A complete clinical examination was done with special reference to the presence of fever, jaundice, bleeding spots, hepatosplenomegaly and to exclude fever with localizing signs such as meningitis, pneumonia, upper respiratory tract infection,skin and subcutaneous tissue infection, etc.

All patients were investigated with complete blood counts, peripheral smear for malarial parasite, chest film, serum biochemistry and urine microscopy.

Peripheral smear positivity was taken as the gold standard for diagnosis of malaria. Other investigations like blood culture, serology for typhoid, urine culture were done where indicated.

Complete blood counts were done by a automated analyzer at the Department of Pathology, BLDEU.

Peripheral blood smear, both thick smears and thin smears were examined by experienced epidemiology department personnel after staining them with JSB (Singh and Bhattacharji stain).

Smear examination was repeated twice in the next 2 days when the patient was febrile before concluding that the illness was non malarious. Malaria was diagnosed when any one of the smears was positive for malarial parasite.

Patient was labeled as having enteric fever if the blood culture was positive for Salmonella typhi or Salmonella paratyphi. Patients with fever, and no localizing findings who had a chest x-ray done which revealed pneumonitis where labeled as having pneumonitis. Urine microscopy was taken as gold standard for diagnosing urinary tract infections.

When all investigations were negative and the patients responded to antipyretics and oral antibiotics the patients were labeled as having viral fever / antibiotic responsive fever.

Patients were labeled as having continuous fever, intermittent fever and remittent fever as per the following definitions.

Continuous fever - The temperature remained above normal throughout the day and did not fluctuate more than 1oC in 24 hours.

Remittent fever - The temperature remained above normal throughout the day with more than 1degree C fluctuation.

Intermittent fever - The fever was present only for some hours of the day and was normal during remaining hours.

RESULTS

Table 3: AGE DISTRIBUTION

Age in years	Number	%
21-30	28	40.0
31-40	15	21.4
41-50	13	18.5
51-60	10	14.2
61-70	4	5.7
Total	70	100.0
Mean ± SD	45 ± 12.25	

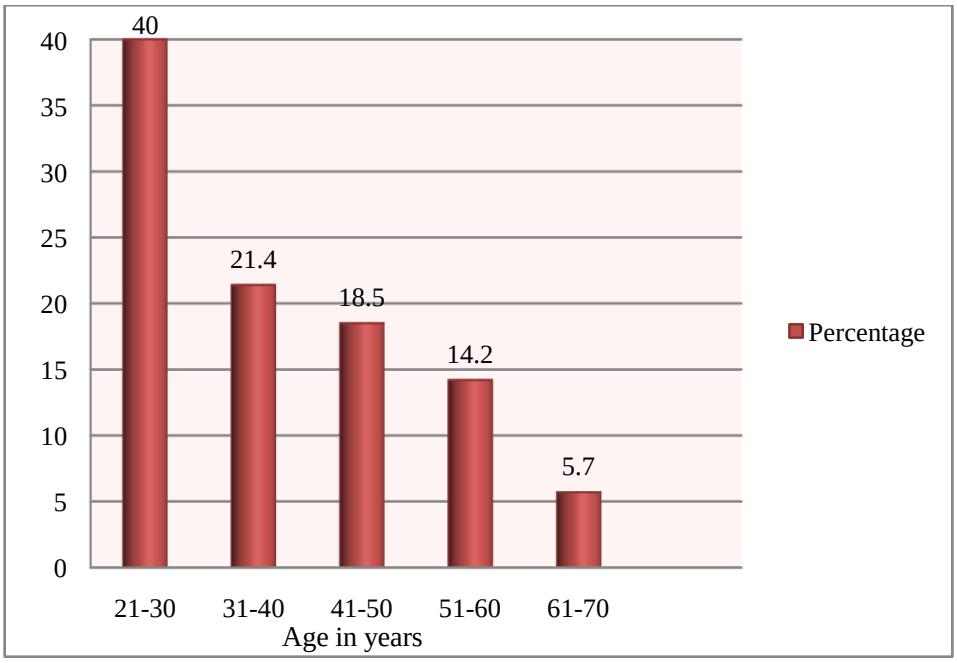


Fig 6: Age Distribution

Table 3: SEX DISTRIBUTION

Sex	Number	%
Male	41	58.5
Female	29	41.4
Total	70	100.0

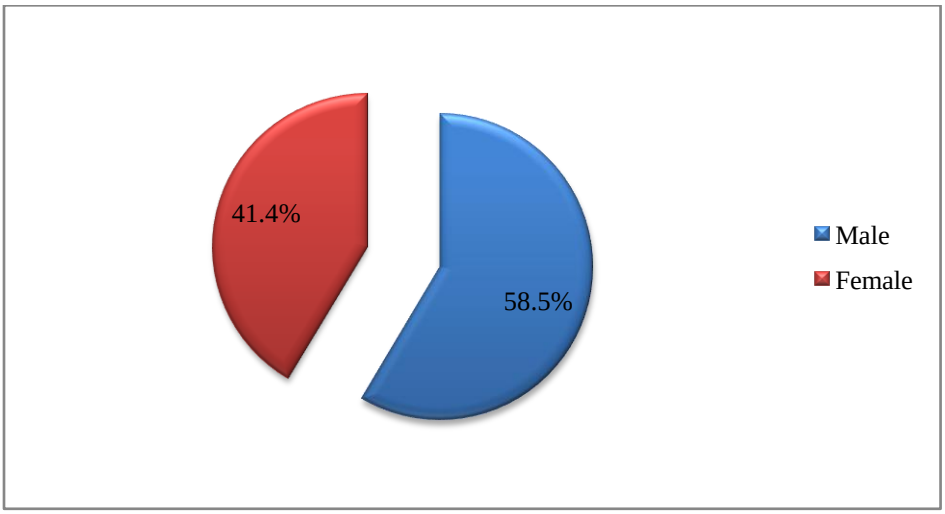


Fig 7: Sex distribution

A total of 70 subjects who diagnosed to have Malaria over a period of two years were studied. The mean age of patients 45± years. The study included 58.5% males and 41.4% females.

Table 5: DISTRIBUTION OF SYMPTOMS

Symptoms	P.Falciparum (n=27)	P.Vivax (n=41)	Mixed infection
1. Typical Paroxysm	14 (51.8%)	28 (68.2%)	2(100%)
2.Vomiting	8 (29.6%)	4 (9.7%)	-
3. Headache	16 (59.2%)	9 (21.9%)	1(50%)
4. Jaundice	14 (51.8%)	8 (19.5 %)	-
5.Altered sensorium	9 (33.3%)	0	1(50%)
6. Pain abdomen	2 (7.4%)	0	-
7. Cough & Breathlessness	4 (14.8%)	2(4.8%)	-
8. Joint pain	0	0	-

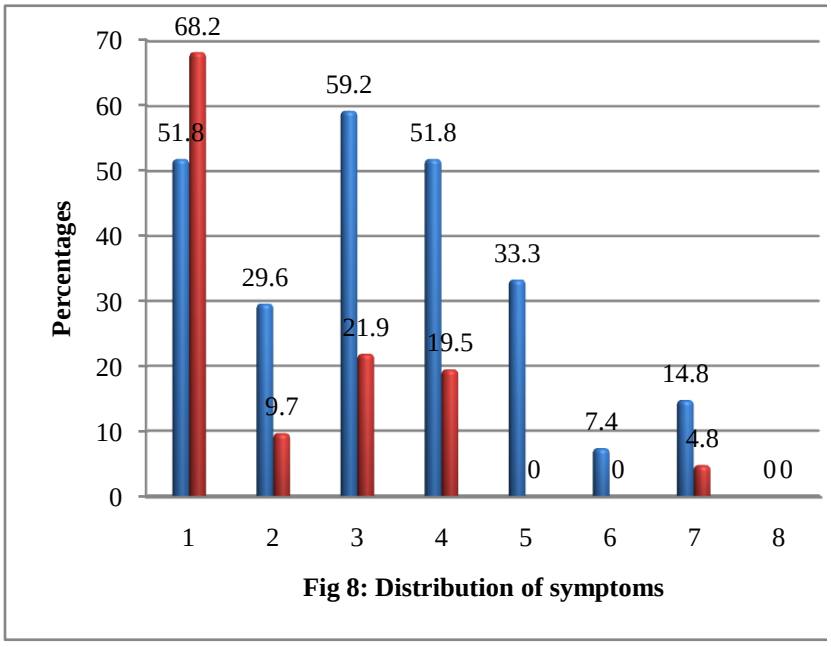


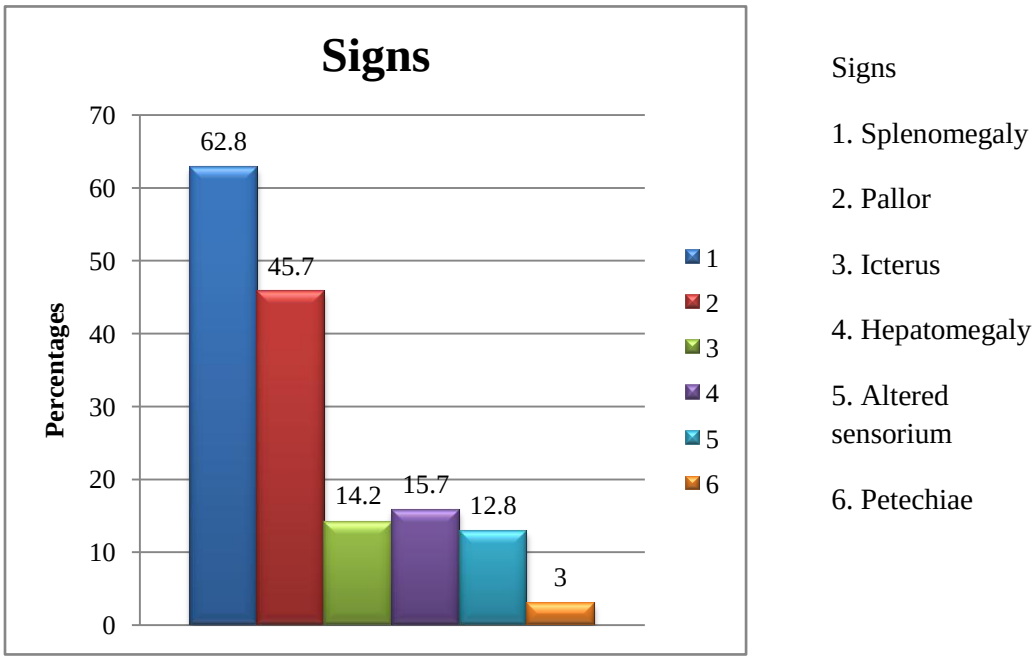
Fig 8: Distribution of symptoms

Typical paroxysms were observered in 28 patients of P.Vivax and 14 patients of P.Falciparum. Under atypical manifestations like vomiting was seen in 8 patients of P.Falciparum and 4 patients in P.Vivax and none in mixed infection, headache in 16 patients of P.Falciparum and 9 in P.Vivax , jaundice in 14 patients

of P. falciparum, 8 P.vivax and none in mixed infection. altered sensorium in 9 patients of P.Falciparum and none in P.Vivax, pain abdomen in 2 patients of P.Falciparum and none in P.Vivax, cough and breathlessness in 4 patients of P.Falciparum and 2 in P.Vivax, no joint pain in P.Falciparum or in P.Vivax. Commonest atypical symptom being headache and vomiting.

Table 6: Distribution of signs

Signs	Number	%
1.Splenomegaly	44	62.8
2.Pallor	32	45.7
3.Icterus	10	14.2
4.Hepatomegaly	11	15.7
5.Altered sensorium	9	12.8
6.Petechiae	2	2.8



Common clinical sign in decreasing order are splenomegaly(62%), pallor(45.7%), Icterus(14.2%), hepatomegaly(15.7%), altered sensorium(12.8%), petechiea(2.8%).

TABLE 7: INCIDENCE OF THROMBOCYTOPENIA

Incidence of thrombocytopenia	Number (n=70)	%
Normal (>1.5 lakh)	9	12.8
Mild (1.0-1.5 lakh)	26	37.1
Moderate (0.50-0.99 lakh)	25	35.7
Severe (<0.5 lakh)	13	18.5

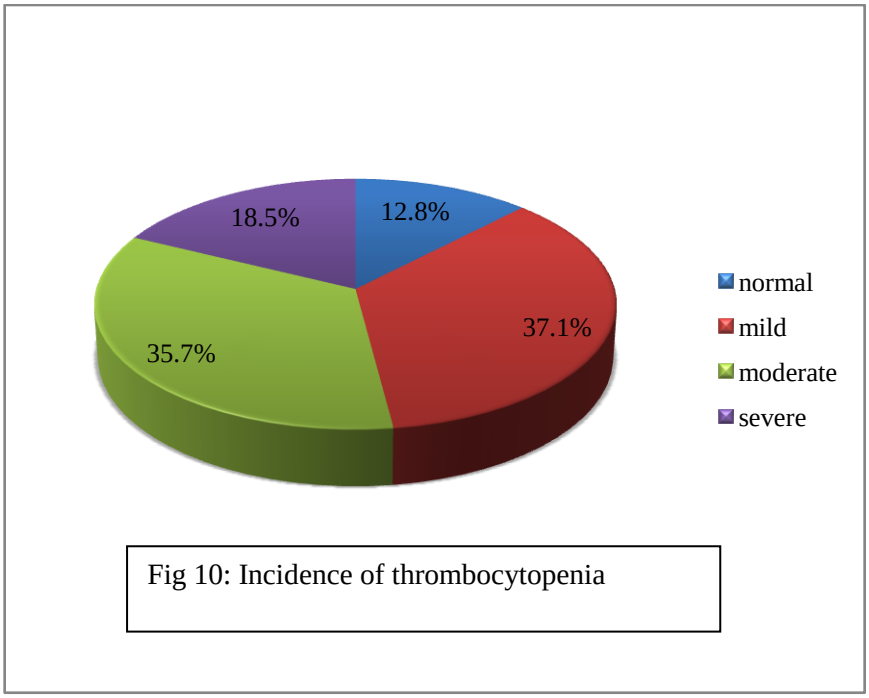


Fig 10: Incidence of thrombocytopenia

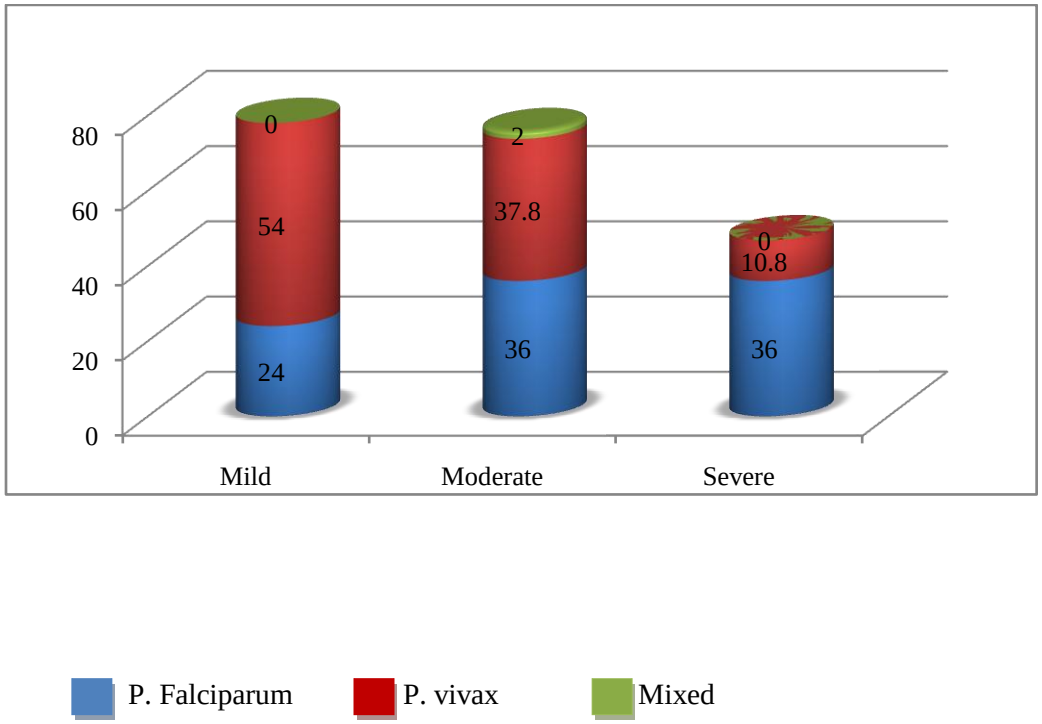
Incidence of Thrombocytopenia was 64(91.4%). with mild Thrombocytopenia 26(37.1%), moderate Thrombocytopenia 25(35.7%) and 13(18.5%) with severe Thrombocytopenia. Normal platelet count was observed in 12.8% of patients ;indicating thrombocytopenia is a common association in malaria.

TABLE 8: TYPE OF SPECIES

Type of species	Number (n=70)	%
P.Falciparum	24	34.3
P.Vivax	38	54.2
Mixed	2	2.8

Table 9: Association of Thrombocytopenia with Species

Thrombocytopenia	Species			Total
Thrombocytopenia	P. Falciparum	P. Vivax	Mixed	Total
Mild	6(24%)	20 (54%)	-	26
Moderate	9(36%)	14 (37.8%)	2(100%)	25
Severe	9 (36%)	4 (10.8%)	-	13
Total	24	38	2	64



Association of Thrombocytopenia with Species

A total of 70 subjects who had malaria, 37 were P.vivax and 25 were P.falciparum .Incidence of P.vivax 52.8% and P.falciparum 35.7 %. 64 out of 70 who had thrombocytopenia were taken up, to study its prognostic implication. Mild Thrombocytopenia were 14(53.8%) in P.Vivax as against to 12(46.1%) in P.Falciparum . Moderate Thrombocytopenia were 14 (37.8%) in P.Vivax as against to 9(36%)in P.Falciparum . Severe Thrombocytopenia were 9(24.3%)in P.Vivax as against to 4 (16%) in P.Falciparum, and in mixed infection 2(100%).

Table 10: Type of Malaria

Type of malaria	Number (n=64)	%
Uncomplicated Malaria	48	75
Complicated Malaria	16	25

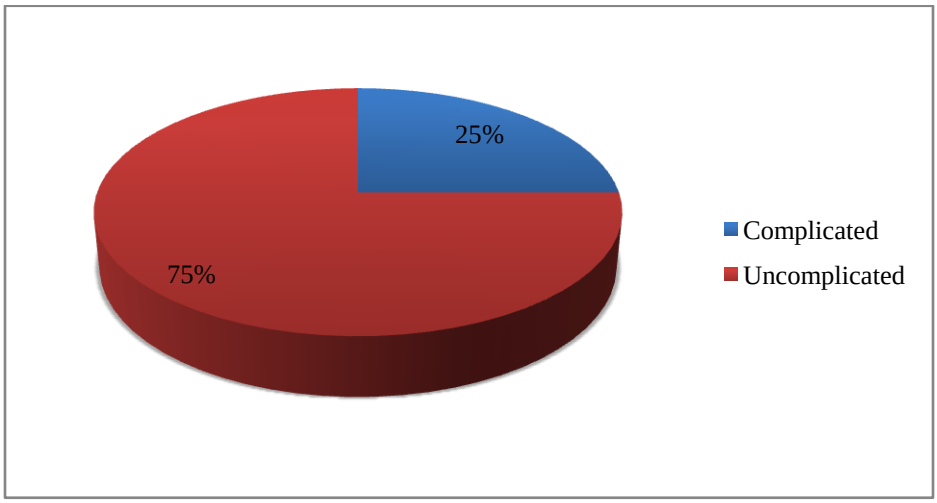


Fig 12: Type of Malaria

Table 11: WHO guidelines for complicated malaria

Criteria	Number of patients	Species		
		P. Falciparum	P.Vivax	Mixed
Hb<5 gm/dl	14	12 (85.7%)	2 (14.2%)	-
S.Creatinine >3mg%	7	4 (57.1%)	2 (28.5%)	1(14.2%)
T.Bilirubin(>3 mg/dl)	11	9 (81.3%)	1(9.1%)	1 (9.1%)
M.acidosis.ph<7.2	12	8 (66.6%)	2(16.6%)	-
Spt bleeding and DIC	1	1(100%)	-	-
Coma >30min	4	4(100%)	-	-
Hyperparasitemia>5%	5	3(60%)	-	2(40%)
B.sugar<40mg%	14	9(64.2%)	4(28.5%)	1(7.4%)
Prostration	12	8(66.7%)	2(16.7%)	2(16.7%)
ARDS	8	6 (75%)	2(25%)	-
Systolic BP<80mmhg	6	3(50%)	3 (50%)	-

According to the revised WHO guidelines of 2000 patients who had Thrombocytopenia were grouped into complicated and uncomplicated. In our study 16 cases had complicated malaria and 54 cases had uncomplicated malaria. In complicated malaria 14 patients had Hemoglobin <5gm% in which 12 (85.7%)were P.Falciparum and 2 (14.2%)were P.Vivax, 7 patients had s.creatinine >3mg% in which 4 (57.1%)were P.Falciparum and 2 (28.5%)were P.Vivax, 11 patients had T.Bilirubin >3mg% in which 9 (81.3%)were P.Falciparum , 1(9.1%)were P vivax and 1(9.1%) mixed; 12 patients had metabolic acidosis (ph<7.2) , 8 (66.6%)were P.Falciparum and 2(16.6%)were P.Vivax,. 1(100%) P.Falciparum patient had spontaneous bleeding with DIC , 4 patients had coma for > 30min, in which all 4(100%) were P.Falciparum, 5 patients had hyperparasitemia in which 3(60%) in

P.Falciparum and 2(40%) in mixed, 14 patients had hypoglycemia in which 9(64.2%) were P.Falciparum, 4(28.5%) were P. vivax, 1(7.4%) was mixed infection. 12 patients had prostration in which 8(66.7%) were P.Falciparum,2(16.7%) P.Vivax and 2(16.7%) mixed, 8 patients had ARDS in which 6 (75%)were P.Falciparum and 2(25%)were mixed, 6 patients developed shock in which 3(50%) were P.Falciparum, and 3(50%) were P.Vivax . Complications were commonly seen in P.falciparum compared to P.vivax of which anemia, hypoglycemia and hyperbilirubinemia being the most common.

Table 12: Association of Thrombocytopenia with severity of malaria

Thrombocytopenia	Severity of malaria		Total
	Uncomplicated	Complicated	
Mild (1.0-1.5 lakh)	24 (50%)	2(12.5%)	26
Moderate (0.5-1.0 lakh)	21(43.7%)	4(25%)	25
Severe (<0.5 lakh)	3(6.3%)	10(62.5%)	13
Total	48 (100.0%)	16(100.0%)	64
Inference	Patients with Severe thrombocytopenia are more likely to have complicated malaria with P<0.003** according to student 'T' test		

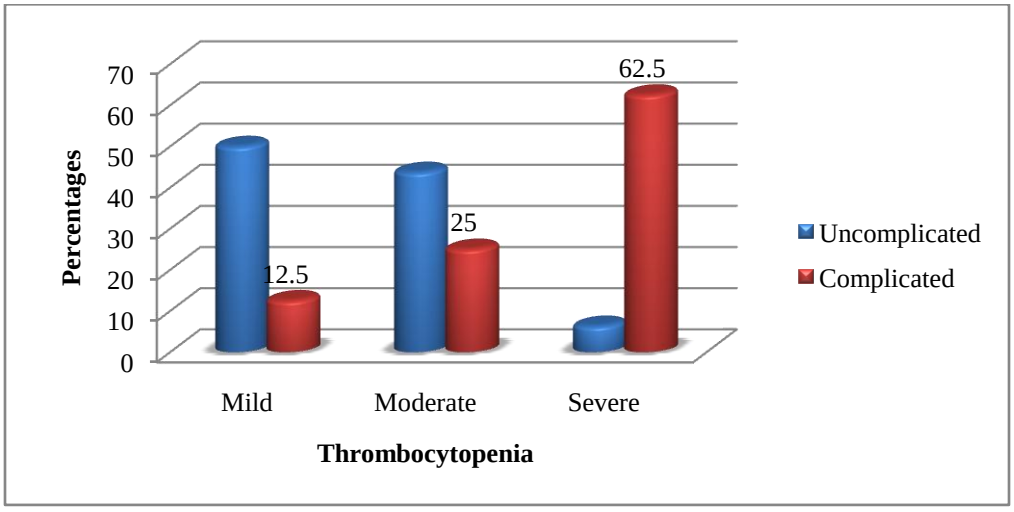


Fig 13: Association of Thrombocytopenia with severity of malaria

Relationship of degree of thrombocytopenia to severity of malaria.Out of 56 uncomplicated malaria, mild thrombocytopenia was noted in 24(42.9%), moderate thrombocytopenia in 24(42.9%), and severe thrombocytopenia in 8(14.3%). Out of 17 cases of complicated malaria mild thrombocytopenia was noted in 3(17.6%), moderate thrombocytopenia in 4(23.5%) and severe thrombocytopenia in 10(58.8%). P value <0.005, was noted in severe thrombocytopenia.

Table 13: Association of Thrombocytopenia with severity of malaria

Species	Type of Malaria		Total	P value
	Uncomplicated	complicated		
P Falciparum	17 (35.4%)	10(62.5%)	27	0. 675
P. Vivax	31(64.5%)	4(25%)	35	0.875
Mixed	-	2 (12.5%)	2	0.05 +
Total	48(100%)	16(100%)	64	0.003

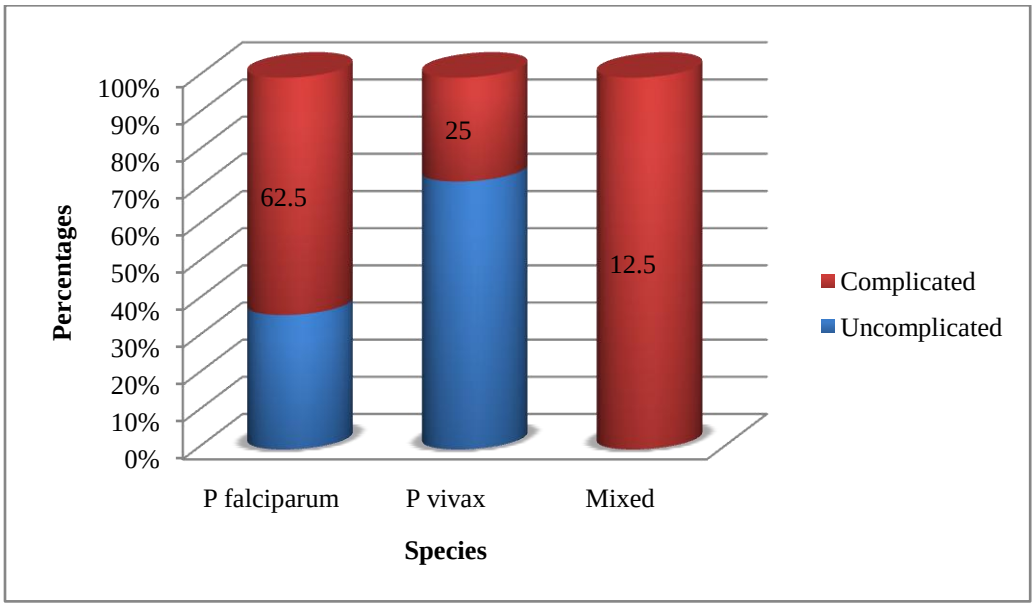


Fig 14: Association of Thrombocytopenia with severity of malaria

Relationship of severity of malaria to species, out of 56 uncomplicated cases of malaria 19(33.9%) were P.Falciparum and 37(66%) were P.vivax. Out of 17 cases of complicated malaria 10(58.8%) were P.falciparum, 5(29.4%) were P.vivax and 2(11.7%) were mixed infection.

Table 14: Number of patients had thrombocytopenia

Platelet counts	Number of patients with thrombocytopenia (n=64)	%
1st day	64	100.0
2nd day	52	81.3
3rd day	44	68.8
4th day	39	60.9
5th day	12	18.8
6th day	3	4.9

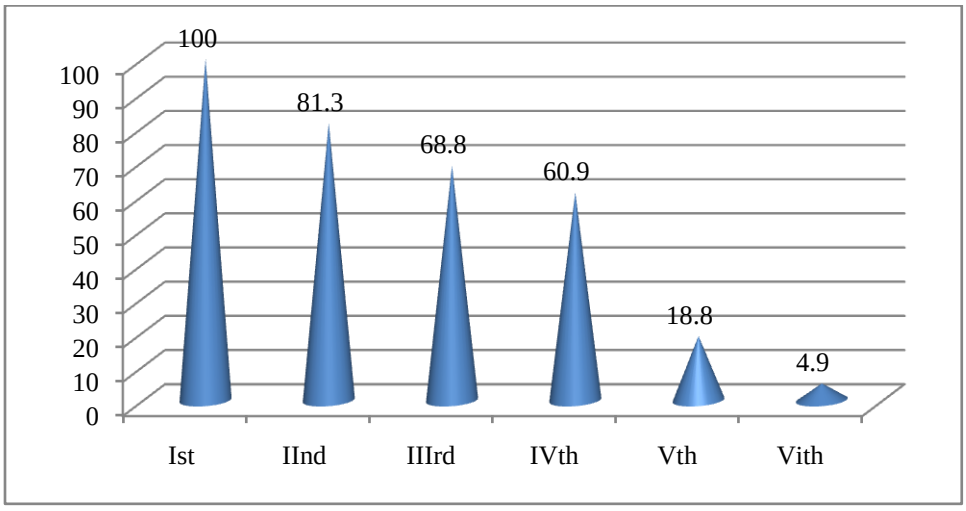


Fig 15: Number of patients had thrombocytopenia

Daily platelet count was done for all patients from the day of admission to day of discharge, and underwent specific treatment. On an average 6th day was considered as last day . On day one 64 patients had low platelet count, on day two 52 patients had low platelet count, on day three 44 patients had low platelet count, on day four 39 patient had low platelet counts, on day five 12 patients had low platelet counts, on day six 3 patients persisted to have low platelet count despite of adequate therapy .

Table 15: Association of Species with outcome

Species	Outcome		Number of patients
	Died	Recovered	
P. Falciparum	1 (3.7%)	26(96.2%)	27
P. Vivax	-	35(100.0%)	35
Mixed	-	2(100.0%)	2
Total	1 (1.5%)	63 (98.4%)	64

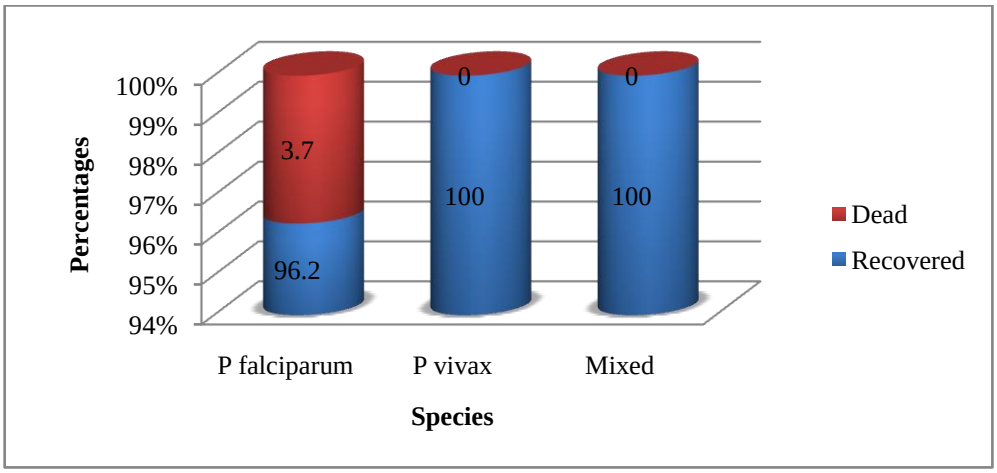


Fig 16: Association of Species with outcome

Out of 64 cases 1 died with the overall mortality of 3.7%.3 patients who persisted to have thrombocytopenia at 6th day 8 recovered and 1 died in which was P.falciparum .

DISCUSSION

A total of 70 malaria cases were studied. Thrombocytopenia was present in 91.4% of the cases in the present study. Thrombocytopenia was present in 100% of the cases with falciparum malaria in our study .

In this study 64 subjects out of 70 malaria cases had thrombocytopenia . Incidence of thrombocytopenia being 91.4% 1. Thrombocytopenia is a common feature of acute malaria and occurs in both P.falciparum and P.vivax infection regardless of severity of infection. Thrombocytopenia in a patient with febrile illness increases the possibility of malarial infection [7]. In this study mild and moderate thrombocytopenia was statistically insignificant when compared to severe thrombocytopenia. We noticed that severe thrombocytopenia was commonly associated with P.falciparum(36%) as compared to P.vivax(10.8%).The mechanism of thrombocytopenia is uncertain. Immune mediated lysis, sequestration in the spleen and a dyspoietic process in the marrow with diminished platelet production have all been postulated. Abnormalities in platelets structure and function have been described as a consequences of malaria, and in rare instances platelets can be invaded by malarial parasite themselves. [99,100] . Thrombopoietin (TPO) is the key factor for platelet production and is elevated in state of platelet depletion. TPO serum levels have been shown to be significantly higher in subjects with severe malaria, normalizing within 14 –21 days of therapy. [101]

Two types of changes in platelet dysfunction are seen in malaria. Initially there is platelet hyperactivity; this is followed by platelet hypoactivity. Platelet hypoactivity results from various aggregating agents like immune complex, surface contact of platelet membrane to malarial red cells damage to the endothelial cells. The injured platelets undergo lysis intravascularly. The release of platelet contents can activate the coagulation cascade and contribute to DIC. Transient hypoactivity is seen following this phase and returns to normal in 1-2 weeks.[99,100]

Thrombocytopenia was present in 91.4% of the cases in the present study. Thrombocytopenia was present in 100% of the cases with falciparum malaria in our study. In a study by Horstmann et al the incidence of thrombocytopenia was 85%59. Sharma S K et al observed that 70% of the patients had thrombocytopenia

[20].Kueh et al had observed that 85% of the patients with falciparum malaria had thrombocytopenia [102]. In our study 86% of the patients with vivax malaria had thrombocytopenia. It was comparable to the study by Horstmann et al where the incidence of thrombocytopenia in vivax malaria was 72%59. In our study 100% of the patients with mixed infection had thrombocytopenia. There are no studies which have mentioned the percentage of thrombocytopenia in mixed infection cases. In our study only 44 patients out of 70 had splenomegaly.It can be observed that only 49% of the patients with thrombocytopenia had splenomegaly.So hereby we can conclude that splenic sequestration is not only the cause of thrombocytopenia other causes such as immune mediated platelet destruction also play a role. In our study there was no difference between the percentage of cases of thrombocytopenia with falciparum and vivax malaria. Similar results were observed by S Looreesuwan et al in their study [56].

In many studies undertaken, the significance of haemostatic abnormalities as a consequences of malaria has been difficult to assess as a result of the presence of various associated complications such as liver dysfunction, uremia.[103,30]. In this study we found that DIC was cause for severe thrombocytopenia in 1 case of P.falciparum. 3 recovered with adequate medical therapy within 7 to 10 days. One patient died of severe metabolic acidosis and multi organ dysfunction [104]. 4 cases of P.vivax malaria had severe thrombocytopenia but none had platelet count less than 20,000. All the 4 cases recovered within one week.64 patients who had thrombocytopenia were categorized into complicated and uncomplicated malaria based on WHO guidelines [56]. Among 64 cases 16(25%) had complicated malaria and 48(75%) had uncomplicated malaria. Among 16 cases of complicated malaria, 10 were P.falciparum, 4 were P.vivax and 2 mixed infection.

Complicated malaria is common in P.falciparum infection [22, 57, 17]. The mechanism for complicated malaria is complex. In P. falciparum infection, membrane protuberance appears on the erythrocyte surface towards the end of the first 24 hrs of asexual cycle. These “knobs” extend high molecular weight antigenically variant, strain specific, adhesive protein (PfEMP1) that mediate attachement to receptors on venules and capillary endothelium an event termed cytoadherence. Several vascular receptors are identified of which intracellular adhesion molecule1 is probably the most important in the brain and CD36 in most other organ. Thus the infected erythrocyte stick inside the small blood vessels. At the same stage, these P.falciparum

infected RBCs may also adhere to uninfected RBCs to form rosettes. The process of cytoadherence, rosetting and agglutination are central to the pathogenesis of P.falciparum malaria.[106]

Among 16 complicated malaria, 14 patients had severe anemia(Hb<5gm%) with hepatic dysfunction and 12(18.8%) had metabolic acidosis [59,62].4 patients had altered sensorium. CSF analysis was done which was normal. 4 patients developed coma and was put on mechanical ventilators, who died within 2 weeks of hospitalization, which shows high mortality rate in cerebral malaria.[58]

Out of 5 complicated P.vivax malaria 2 had severe anemia(Hb<5gm%), 3 developed ARF secondary to severe vomiting and dehydration, Renal impairments is common among adults with severe P.falciparum malaria. Studies also suggested that P.vivax can also cause renal dysfunction. [107, 89.] . The pathogeneses of renal failure is unclear but may be related to erythrocyte sequestration interfering with renal microcirculatory flow and metabolism. Clinically and pathologically this syndrome manifests as acute tubular necrosis, although cortical necrosis never develops. In survivors, urine flow resumes in a median of four days, and serum creatinine levels return to normal in a mean of 14 days. [106]

In this study all the three patients who developed ARF, their serum creatinine returned to normal by 8th day. One patient required dialysis and other two were treated conservatively.[70, 89]. There were no deaths in complicated vivax malaria.

When thrombocytopenia is co-related with severity of malaria, severe thrombocytopenia was commonly associated with complicated malaria(58.8 %) as compared to uncomplicated malaria(23.5 %) [12] . Maximum thrombocytopenia occurred on third and fourth day of infection and gradually returned to normal by fifth to sixth day [45]. Those persisted to have severe thrombocytopenia beyond 6th day, their mortality and morbidity increased despite of adequate therapy.

Patients who had severe thrombocytopenia at the time of admission are 8.5 times more prone to develop complications when compared to mild and moderate thrombocytopenia based on student ‘T’ test. In this study 12 patients had severe thrombocytopenia beyond 6th day, 8 recovered within 7 to 10 days, 1 died which was P.falciparum malaria increasing mortality rate from 4.1% to 10%.

SUMMARY

Malaria continues to be a huge social, economical and health problem, particularly in the tropical countries. Complicated malaria is a major cause for morbidity and mortality.

Early diagnosis and prompt treatment of complications reduces the global burden of malaria. Severity of thrombocytopenia is a better predictor of outcome but it does not help in early diagnosis of complicated malaria. Hence further studies should be conducted on thrombocytopenia in malaria, rate of fall in platelet count which may help in early diagnosis of complications in malaria.

CONCLUSION

Thrombocytopenia is a common association of Malaria. Severe thrombocytopenia (platelet count<20,000) is seen in P.falciparum, uncommon in P.Vivax malaria. Severe thrombocytopenia is a good predictor of poor prognosis than mild and moderate thrombocytopenia. Patients who present with severe thrombocytopenia are 8.5 times more prone to develop complications than mild and moderate thrombocytopenia. If severe thrombocytopenia persists for more than six days despite of adequate therapy, mortality rate increases from 4.1% to 10%.

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ANNEXURE

CONSENT FORM

TITLE OF RESEARCH : STUDY OF THROMBOCYTOPENIA IN MALARIA-CORRELATION WITH TYPE AND SEVERITY OF MALARIA AND ITS PROGNOSTIC SIGNIFICANCE

GUIDE : DR. R.C. BIDRI

P.G. STUDENT : DR. NIJORA DEKA

PURPOSE OF RESEARCH:

I have been informed that the purpose of this study is to evaluate the relationship between the platelet count and the type and severity of malaria.

PROCEDURE:

I understand that I will undergo detailed history and clinical examination after which blood will be collected & sent to the laboratory for investigations.

RISKS AND DISCOMFORTS:

I understand that there is no risk involved and I may experience mild pain during the collection of blood.

BENEFITS:

I understand that my participation in this study will help in recognition of existence of relationship between platelet count and malaria that will ultimately benefit my fellow beings.

CONFIDENTIALITY:

I understand that the medical information produced by the study will become a part of hospital record and will be subjected to confidentiality and privacy regulations of hospital. If the data is used for publications, the identity of the patient will not be revealed.

REQUEST FOR MORE INFORMATION:

I understand that I may ask for more information about the study at any time.

REFUSAL OR WITHDRAWAL OF PARTICIPATION:

I understand that my participation is voluntary and I may refuse to participate or withdraw for study at any time.

INJURY STATEMENT:

I understand in the unlikely event of injury to me during the study I will get medical treatment but no further compensations.

(Signature of Guardian) (Signature of patient)

(If the patient is conscious, well oriented and fully aware)

PROFORMA

Name: IP. No:

Age: Address:

Sex: Date of Admission:

Occupation: Date of Discharge:

Religion: Status at Discharge:

PRESENTING COMPLAINTS:

Typical paroxysm

Fever - Duration

Onset: Sudden / Insidious

H/o Chills and rigors – Present / Absent

Sweating – Present / Absent

Diurnal variation – Present / Absent

Type of Fever – Continuous / intermittent / remittent

Atypical symptoms

Jaundice: Duration :

High coloured urine – Present / Absent

Clay coloured stools – Present / Absent

Pruritus – Present / Absent

Nausea – Present / Absent

Bleeding: (signs of Thrombocytopenia)

Skin rashes – Present / Absent

Haematuria – Present / Absent

Malena – Present / Absent

Haematemesis – Present / Absent

Epistaxis – Present / Absent

Gum bleeding – Present / Absent

Vomiting of blood

Coughing of blood

Pain in abdomen:

Duration:

Site:

Nature:

Radiation:

Aggravating factors:

Relieving factors:

Altered sensorium :

Onset : Sudden / insidious

Convulsions : Yes/no

Others if any:

HISTORY OF PRESENTING ILLNESS:

PAST HISTORY:

History of malaria in the past :

PERSONAL HISTORY:

Appetite

Diet

Sleep

Bowel and Bladder

Habits

OBSTETRIC & MENSTRUAL HISTORY:

FAMILY HISTORY:

GENERAL PHYSICAL EXAMINATION:

Height:

Weight:

Appearance :healthy /ill/toxic

Built

Nourishment

Pallor:

Icterus:

Cyanosis:

Clubbing:

Pedal edema:

Lymphadenopathy:

Petechiae

Purpura

Vital Signs:

Pulse rate :

Blood pressure :

Temperature :

Respiratory rate :

GASTROINTESTINAL SYSTEM

Inspection : Shape

Movement with respiration:

Visible peristalsis: Present / Absent

Engorged veins: Present / Absent

Position of umbilicus:

Scar Marks:

Palpation : LIVER - Palpable by--------cms below RCM / Not palpable

Consistency: soft / firm /irregular

Tenderness: present/absent

Margin: regular /irregular

Movement with respiration: present/absent

SPLEEN: Palpable by--------cms below LCM / Not palpable

Consistency: soft / firm /irregular

Tenderness: present/absent

Margin: regular /irregular

Movement with respiration: present/absent

Percussion : Free Fluid present/absent

Auscultation :

CARDIOVASCULAR SYSTEM :

RESPIRATORY SYSTEM :

CENTRAL NERVOUS SYSTEM :

COMPLICATIONS OF MALARIA :

Impaired consciousness :

Prostration :

Jaundice :

Cerebral malaria :

Generalised convulsions :

Severe anemia :

Renal failure :

Hypoglycemia :

Fluid electrolyte, acid base disturbances :

Pulmonary oedema :

Algid malaria :

DIC :

Hyperpyrexia :

PROVISIONAL DIAGNOSIS :

INVESTIGATIONS

HAEMATOLOGY

Haemoglobin	gm/dl
TC	Cells/mm^3
DC	
Neutrophils	%
Lymphocytes	%
Eosinophils	%
Basophils	%
Monocytes	%
ESR	mm/1hr
Platelet count	cells/cumm
Peripheral smear study for malarial parasite	
QBC test	
Antigen Test	
Dengue IgG/ IgM	
Leptospiral IgG/IgM	

URINE

Albumin	
Sugar	
Microscopy	

BIOCHEMISTRY

RBS	mg/dl
FBS	mg/dl
PPBS	mg/dl

RENAL FUNCTION TESTS

Urea	mg/dl
Creatinine	mg/dl
Sodium	meq/L
Potassium	meq/L

LIVER FUNCTION TESTS

Total bilirubin	mg/dl
Conjugated bilirubin	mg/dl
Unconjugated bilirubin	mg/dl
Total protein	gm/dl
Albumin	gm/dl
A/G ratio	

SGOT	IU/L
SGPT	IU/L
ALP	IU/L

ELECTROCARDIOGRAPHY

CHEST X-RAY

WHO guidelines for complicated malaria

Course in the hospital	Day 1	Day 2	----	-----
Hb<5 gm/dl				-
S.Creatinine				
T.Bilirubin(>3 mg/dl)				
M.acidosis.ph<7.2				
Spt bleeding				
Coma >30min				
Hyperparasitemia>5 %				
B.sugar<40mg%				
Prostration				
ARDS				
Systolic BP<80mmhg				

DIAGNOSIS

MASTER CHART CASES

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
1	20596	50	F	Y	+	-	+	+	+	-	-	-	-	-	+	N	N	V	11.2	11200	64	34	06	00	02
2	6230	26	F	Y	+	-	+	+	-	-	-	-	-	-	-	N	N	F	11.2	6800	68	42	04	00	02
3	15780	20	F	Y	+	+	-	-	+	+	-	+	-	+	+	N	Y	V	9.6	19700	90	07	03	00	00
4	12155	40	M	Y	+	+	-	-	-	+	-	-	-	-	+	N	N	M	7.9	4500	53	44	03	00	00
5	12189	35	F	Y	+	+	-	-	-	-	-	-	-	-	+	N	N	V	9.7	3400	68	30	02	00	00
6	1654	55	F	Y	+	+	-	-	+	-	-	-	-	-	+	N	N	V	8.5	4700	48	45	05	00	00
7	35642	42	F	Y	+	-	+	-	+	-	-	+	-	+	-	N	N	F	10.4	6800	78	20	02	00	00
8	13230	70	F	Y	-	+	-	+	-	-	-	-	-	-	+	N	N	V	12.9	5400	80	15	05	00	00
9	3162	38	F	Y	-	+	+	-	-	-	-	-	-	-	+	N	N	F	10.2	10800	58	44	03	00	00
10	9567	60	M	Y	+	+	-	+	-	+	-	+	-	-	+	N	N	F	9.8	4000	72	24	04	00	00
11	15966	25	M	Y	+	+	-	-	+	-	-	-	-	-	-	N	N	V	12.5	7700	74	24	02	00	00
12	7292	24	M	Y	+	-	+	-	-	-	-	+	-	-	-	N	N	M	12.4	4400	55	40	04	00	01
13	15957	45	F	Y	-	+	+	-	+	-	-	-	-	-	-	N	N	V	5.9	1100	55	43	02	01	00
14	16586	65	M	Y	-	+	-	-	-	+	-	-	-	-	+	N	C	F	5.2	7500	47	44	02	00	00
15	17059	28	M	Y	+	-	+	+	+	+	-	-	-	-	+	N	N	F	13.4	4600	80	16	04	00	00
16	19129	88	M	Y	+	-	+	-	+	+	-	-	-	-	+	N	N	V	11.9	3300	79	17	03	01	00
17	17691	50	M	Y	-	+	+	-	-	+	-	-	-	-	-	N	I	V	11	5200	70	24	02	04	00
18	14988	60	F	Y	-	+	+	-	-	-	-	-	-	-	+	N	N	V	8.1	9000	86	12	02	00	00
19	15550	26	M	Y	-	+	-	-	-	-	-	-	-	-	-	N	N	V	15.6	3800	36	60	04	00	00
20	6182	52	F	Y	+	+	-	-	-	-	-	-	-	-	+	N	N	F	10.1	11600	70	27	03	00	00
21	3626	50	F	Y	+	+	-	-	-	-	-	+	-	-	-	N	N	F	10.8	4600	62	34	04	00	00
22	13020	20	M	Y	+	-	+	-	-	-	+	+	-	-	-	N	N	F	12.1	8800	51	47	02	00	00
23	13130	60	M	Y	+	+	+	-	+	+	-	-	+	+	-	N	C	V	10.4	4300	85	30	02	00	00
24	6934	26	M	Y	-	-	+	-	-	-	-	-	-	-	+	N	N	V	12.1	3400	70	23	04	00	00
25	8635	45	M	Y	+	+	-	-	+	+	-	-	-	-	+	N	C	F	11.8	6800	78	20	01	01	00
26	12090	50	M	Y	+	+	-	-	+	-	-	-	-	+	-	N	C	F	12.7	4400	77	21	02	00	00
27	14027	22	M	Y	+											N	N	F	14.4	3100	88	10	02	00	00
28	15520	27	M	Y	-	+	-	-	-	-	-	-	-	-	-	N	N	V	12.7	4000	72	24	03	01	00
29	13641	25	F	Y	-	+	-	-	+	-	-	-	-	-	-	N	N	F	10	14600	91	5	4	00	00
30	13371	21	M	Y	-	+	-	-	-	-	-	-	-	-	-	N	N	VV	10.4	7400	54	38	05	03	00
31	8628	30	M	Y	-	+	-	-	-	-	-	-	-	-	-	N	I	F	10.9	2600	60	36	04	00	00
32	26824	28	F	Y	-	+	+	+	-	-	-	-	+	+	-	N	N	V	9.8	11300	78	28	03	00	00
33	5056	60	M	Y	+	-	+	-	-	-	-	-	-	-	-	N	N	F	11.6	11600	46	54	04	02	03
34	12671	35	F	Y	-	+	-	-	-	+	-	-	+	+	+	Y	N	V	10.2	10200	74	24	02	00	00
35	1137	45	F	Y	-	+	-	-	-	-	-	-	-	+	-	N	N	F	5.2	20000	84	14	02	00	00
36	14126	45	F	Y	+	-	+	-	-	-	-	-	-	-	-	N	N	V	10.2	10800	64	34	02	00	00
37	6160	65	M	Y	-	+	-	-	+	-	-	-	-	-	-	N	N	V	10.8	10800	84	16	00	00	00
38	11705	38	F	Y	+	-	+	+	-	-	-	-	-	-	-	N	N	V	10.2	10600	68	32	00	00	00
39	12824	70	F	Y	+	+	-	-	-	-	-	-	-	-	+	N	N	F	12.6	11000	64	36	00	00	00
40	3958	40	F	Y	-	+	-	-	-	-	-	-	-	-	-	N	N	V	13.2	11000	54	46	00	00	00
41	7093	40	M	Y	+	+	-	-	-	-	-	-	-	-	-	N	N	V	9.8	12000	54	42	03	01	00
42	12248	30	M	Y	+	-	+	-	-	-	-	-	-	-	-	N	N	V	12.4	10800	68	29	03	00	00
43	4445	26	M	Y	+	+	-	-	-	-	-	-	-	-	+	N	N	V	11.2	11002	64	36	00	00	00
44	21104	55	M	Y	+	+	-	-	-	-	-	-	-	-	-	N	N	F	12.6	11800	84	16	00	00	00
45	1936	26	F	Y	+	+	-	-	-	-	-	-	-	-	-	N	N	V	11.6	10800	64	36	00	00	00
46	21708	36	F	Y	-	+	-	-	+	+	-	+	-	-	+	N	N	V	12	11000	64	36	00	00	00
47	22186	28	F	Y	-	+	+	-	+	+	-	-	-	+	-	N	N	V	12.1	11800	76	24	00	00	00
48	22806	35	M	Y	+	-	+	-	-	+	-	-	-	-	+	N	N	V	12.2	11800	74	26	00	00	00
49	12195	52	F	Y	-	+	+	-	+	+	-	-	-	-	+	N	N	V	11.2	10600	76	24	00	00	00
50	3599	48	M	Y	-	+	-	-	-	-	-	-	-	-	-	N	N	V	11.6	10800	86	14	00	00	00
51	23412	32	M	Y	-	-	-	-	-	-	-	-	-	-	-	N	N	F	12.2	11200	84	16	00	00	00
52	28066	40	M	Y	+	-	-	+	+	-	+	+	+	-	-	N	N	F	12.2	11600	84	16	00	00	00
53	26529	42	F	Y	+	-	-	-	-	-	-	+	+	-	-	N	N	F	9.2	10800	82	18	00	00	00
54	13102	35	F	Y	-	-	-	-	-	+	-	+	+	-	-	N	N	F	9.8	12800	68	42	00	00	00
55	6289	28	F	Y	+	-	+	-	-	+	-	-	-	-	-	N	N	V	12.2	11800	78	22	00	00	00
56	14358	28	M	Y	+	-	+	-	-	+	-	-	-	-	-	N	N	F	10.2	10800	62	32	04	00	02
57	12626	27	M	Y	+	-	+	-	-	+	-	-	-	-	-	N	N	F	13.6	12200	56	49	03	00	02
58	8007	33	M	Y	+	-	-	-	-	-	-	-	-	+	+	N	N	V	12.4	11200	62	38	00	00	00
59	11392	22	M	Y	+	+	-	+	-	-	-	-	-	+	+	N	N	V	12.8	11800	74	28	06	00	00
60	14752	60	F	Y	-	+	-	+	-	+	-	-	-	+	-	N	N	V	10.2	9600	84	16	00	00	00
61	12893	32	M	Y	+	+	-	+	+	+	+	-	-	+	-	N	N	F	12.2	11800	64	36	00	00	00
62	19073	40	M	Y	-	+	+	+	+	+	+	-	+	+	-	N	N	V	9.2	10800	64	36	00	00	00
63	34979	42	M	Y	+	+	+	-	-	-	-	+	+	-	-	N	N	F	9.8	11000	76	24	00	00	00
64	48390	35	M	Y	+	-	-	-	-	-	+	+	-	-	+	N	N	V	12.2	11800	84	16	00	00	00

1	27 1st	2nd	3rd	4th	5th	6th	28	29	30	31	32	33	34	35	36	37	38	39
1	112000	150000	150000	180000	200000	220000	20	N	N	-	N	-	-	-	-	-	Q,p	Imp
2	116000	130000	120000	120000	160000	200000	12	N	N	-	N	-	-	-	-	-	Q	Imp
3	98000	100000	950000	100000	135000	185000	50	N	N	+	N	-	+	-	-	+	Q,p	Imp
4	100000	820000	100000	120000	180000	200000	30	I	I(3.4)	-	N	+	+	+	-	-	Q	Imp
5	97000	100000	980000	180000	200000	280000	10	N	I (11.8)	-	N	-	+	+	+	+	Q,p	Imp
6	103000	100000	120000	180000	200000	200000	25	N	N	-	N	-	-	-	-	-	Q,p	Imp
7	94000	960000	180000	200000	220000	230000	36	N	N	+	N	-	-	+	-	-	Q	Imp
8	47000	540000	840000	900000	120000	190000	70	N	N	-	N	-	-	-	-	-	Q,p	Imp
9	103000	104000	140000	160000	210000	220000	35	N	N	-	N	-	-	-	-	-	A	Imp
10	65000	80000	740000	100000	220000	240000	75	N	N	+	N	+	-	+	-	-	Q	Imp
11	151000	170000	180000	170000	280000	260000	10	N	N	-	N	-	-	-	-	-	Q, p	Imp
12	87000	880000	100000	120000	109000	220000	40	I	N	-	N	+	-	+	-	-	Q,p	Imp
13	115000	103000	170000	200000	204000	209000	50	N	N	-	N	-	-	-	-	-	Q,p	Imp
14	33000	40000	540000	620000	800000	940000	30	I	I (5)	+	N	-	+	+	+	+	Q	Imp
15	81000	94000	120000	109000	134000	190000	45	I	I (4)	-	N	-	-	-	+	-	Q	Imp
16	11000	36000	500000	89000	120000	230000	25	I	N	+	N	-	+	+	-	+	Q,p	Imp
17	43000	54000	68000	100000	120000	160000	20	I	N	+	N	-	+	-	+	-	Q,p	Imp
18	102000	100000	220000	230000	232000	230000	50	N	N	-	N	-	-	-	-	-	Q,p	Imp
19	30000	90000	108000	150000	180000	210000	15	N	N	-	N	-	-	-	-	-	Q,p	Imp
20	80000	94000	120000	109000	134000	190000	20	N	N	+	N	-	+	-	+	-	Q	Imp
21	59000	54000	840000	900000	120000	190000	30	I	N	+	N	-	-	-	-	-	Q	E
22	30000	40000	540000	620000	800000	940000	20	N	I (8)	+	N	-	+	+	+	-	A	Imp
23	40000	94000	120000	109000	134000	190000	25	N	N	-	N	-	-	-	-	-	Q,p	Imp
24	83000	83200	840000	900000	120000	190000	10	N	N	-	N	-	-	-	-	-	A	Imp
25	40000	40000	540000	620000	800000	940000	48	N	I (3.7)	+	N	-	+	-	-	-	Q	Imp
26	50000	94000	120000	109000	134000	190000	45	N	N	+	N	+	+	+	+	+	Q	E
27	80000	94000	120000	109000	134000	190000	30	N	N	+	N	-	-	-	-	-	Q	Imp
28	100000	154000	184000	190000	190000	190000	35	N	N	-	N	-	-	-	-	-	Q,p	Imp
29	85000	94000	120000	109000	134000	190000	25	N	N	-	N	-	-	-	-	-	Q	Imp
30	43000	54000	90000	150000	190000	203000	85	N	N	-	N	-	-	-	-	-	Q,p	Imp
31	54000	94000	120000	109000	134000	190000	22	I	I(5.8)	-	N	-	-	-	-	-	Q	Imp
32	116000	100600	120000	180000	200000	200000	25	N	N	-	N	-	-	-	-	-	Q,p	Imp
33	187000	196000	180000	200000	220000	230000	32	N	N	-	N	-	-	-	-	-	A	Imp
34	99000	99000	120000	109000	134000	190000	32	N	N	-	N	-	-	-	-	-	Q,p	Imp
35	86000	100000	120000	180000	200000	200000	12	I	I (3.1)	-	N	-	+	+	+	+	Q	Imp
36	108000	100000	120000	180000	200000	200000	25	N	N	-	N	-	-	-	-	-	Q,p	Imp
37	108000	960000	180000	200000	220000	230000	36	N	N	-	N	-	-	-	-	-	Q,p	Imp
38	108000	100000	120000	180000	200000	200000	28	N	N	-	N	-	-	-	-	-	Q,p	Imp
39	97000	960000	180000	200000	220000	230000	44	N	N	-	N	-	-	-	-	-	Q	Imp
40	101000	100000	120000	180000	200000	200000	11	N	N	-	N	-	-	-	-	-	Q,p	Imp
41	184000	960000	180000	200000	220000	230000	44	N	N	-	N	-	-	-	-	-	Q,p	Imp
42	186000	170000	180000	170000	280000	260000	56	N	N	-	N	-	-	-	-	-	Q,p	Imp
43	106000	880000	100000	120000	109000	220000	25	N	N	-	N	-	-	-	-	-	Q,p	Imp
44	151000	103000	170000	200000	200000	209000	44	N	N	-	N	-	-	-	-	-	Q	Imp
45	102000	170000	180000	170000	280000	260000	56	N	N	-	N	-	-	-	-	-	Q,p	Imp
46	151000	880000	100000	120000	109000	220000	44	N	N	-	N	-	-	-	-	-	Q,p	Imp
47	108000	103000	170000	200000	200000	209000	32	N	N	-	N	-	-	-	-	-	Q,p	Imp
48	167000	170000	180000	170000	280000	260000	32	N	N	-	N	-	-	-	-	-	Q,p	Imp
49	104000	1038000	100000	120000	109000	220000	45	N	N	-	N	-	-	-	-	-	Q,p	Imp
50	120000	113000	170000	200000	200000	209000	28	N	N	-	N	-	-	-	-	-	Q,p	Imp
51	166000	170000	180000	170400	280000	260000	64	N	N	-	N	-	-	-	-	-	Q	Imp
52	87000	880000	100000	120000	109000	220000	36	N	I (4)	+	N	-	+	+	-	-	Q	Imp
53	104000	100000	120000	180000	200000	200000	28	N	N	+	N	-	-	-	-	-	A	Imp
54	47000	960000	180000	200000	220000	230000	54	N	I (3.2)	+	N	-	+	-	-	-	Q	Imp
55	182000	100000	120000	180000	200000	200000	45	N	N	-	N	-	-	-	-	-	Q,p	Imp
56	106000	100000	120000	180000	200000	200000	22	N	N	-	N	-	-	-	-	-	Q	Imp
57	98000	94000	120000	109000	134000	190000	12	N	N	-	N	-	-	-	-	-	Q	Imp
58	97600	84000	840000	900000	120000	190000	14	N	N	-	N	-	-	-	-	-	Q,p	Imp
59	82000	40000	540000	620000	800000	940000	12	N	N	-	N	-	-	-	-	-	Q,p	Imp
60	117000	94000	120000	109000	134000	190000	25	N	N	-	N	-	-	-	-	-	Q,p	Imp
61	45000	94000	120000	109000	134000	190000	44	N	I(6)	-	N	-	+	-	-	-	Q	Imp
62	127000	960000	180000	200000	220000	230000	56	N	N	-	N	-	-	-	-	-	Q,p	Imp
63	113000	254000	190000	184000	190000	203000	25	N	N	-	N	-	-	-	-	-	Q	Imp
64	105000	224000	220000	209000	234000	290000	44	N	N	-	N	-	-	-	-	-	Q,p	Imp

Key to Master Chart

1. Serial no
2. IP no
3. Age in years
4. Sex

M = Male F = Female

5. Fever

+ = Yes - = No

6. Chills and rigors

+ = Yes - = No

7. Nausea and vomiting

+ = Yes - = No

8. Easy fatiguability

+ = Yes - = No

9. Abdominal pain

+ = Yes - = No

10. Altered Sensorium

+ = Yes - = No

11. Pallor

+ = Mild ++ = Moderate +++ = Severe

12. ICT = Icterus

+ = Present - = Absent

13. Pedal = Pedal oedema

+ = Present - = Absent

14. Spleen

+ = Palpable - = Not palpable

15. Hepatomegaly

+ = Present - = Absent

16. RS = Respiratory system

N = Normal R = Ronchi C = Crepitation

17. CVS = Cardiovascular system

N = Normal M = Murmur

18. CNS = Central nervous system

N = Normal I = Impaired consciousness C = Coma > 30min

19. SPE = species

M = Mixed V = Vivax F = Falciparum

20. Hb% = Hemoglobin < 5%

21. TC = Total count (per cumm)

22. Differential count Neutrophils %

23. Differential count Lymphocytes %

24. Differential count Eosinophils %

25. Differential count Monocytes %

26. Differential count Basophils %

27. Plt = platelet count (lakhs /cumm)

28. ESR = Erythrocyte sedimentation rate (at the end of 1 hour)

29. RFT = Renal function test s. creat > 3mg%

N = Normal I = impaired

30. Bil = Bilirubin > 3mg/dl

N = Normal I = Impaired

31. Metabolic acidosis ph < 7.2

+ = Yes - = No

32. CXR = Chest x-ray

N = Normal C = COPD changes PE = Pulmonary oedema

33. Hyperparasitemia >5 %

+ = Yes - = No

34. B.sugar<40mg%

+ = Yes - = No

35. Prostration

+ = Yes - = No

36. ARDS

+ = Yes - = No

37. Systolic BP<80mmhg

+ = Yes - = No

38. Rx = Treatment

AS = Artesunate AE = Arteether C = Chloroquine Q = Quinine D = Doxycycline

P = primaquine

39. Outcome

Imp- Improved E- Expired