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MALARIA
(at the Chris Hani/Baragwanath Hospital)

AN "ALIEN" EPIDEMIC ?

Dr Chrissoula Teresa Gavalakis

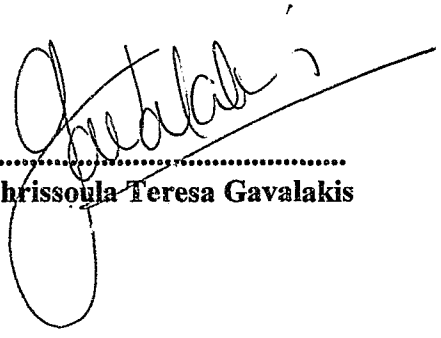
**A dissertation submitted to the Faculty of Medicine,
University of the Witwatersrand,
in partial fulfilment of the requirements for
the degree of *Master of Medicine* in the branch of Haematology.**

**Johannesburg
1998**

Ethics approval was obtained from the
Committee for Research on Human Subjects (Medical)
University of Witwatersrand.
Clearance Certificate No. M 940704
(Ref: R14/49 Gavalakis)

DECLARATION

I declare that this dissertation, apart from the laboratory investigations performed on the blood samples taken from the patients in this study, is my own unaided work. It is being submitted for the degree of *Master of Medicine* (in the branch of Haematology) to the University of Witwatersrand. It has not been submitted for any degree or examination in any other University.



.....

Dr Chrissoula Teresa Gavalakis

June 1998

DEDICATION

To my daughter, *Alexandra Aglaia Voulgaris*
and to my husband, *John*

ABSTRACT

Despite the efforts, for more than twenty years, to control malaria, the incidence of this disease still appears to be escalating globally. At the Chris Hani/Baragwanath Hospital, data analysis of malaria admissions between January 1994 to December 1996 showed an increasing trend from year to year.

The main objective of the study was to try and provide some insight into this increasing rate of malaria at the Chris Hani/Baragwanath Hospital. The study was structured into two main parts: a retrospective analysis which concentrated on malaria admissions between and including January 1994 and March 1994, and a prospective analysis interviewing and examining all the malaria cases that were diagnosed between and including January 1995 and March 1995. Both aspects of the study assessed the patients socioeconomically, haematologically and immunologically. A detailed travel,medical and drug history was taken through the aid of a questionnaire.

Two hundred and sixty-three patients (175 male and 88 female), of which 35% (91/263) were children (< 13 years old), were diagnosed with malaria. The clinical and laboratory presentations were consistent with other studies. The prevalence of complicated disease however was less than what has been described in the literature; cerebral malaria (as defined by Warrell, 1982) was documented in 1% of patients, hypoglycaemia (glucose < 2.2 mmol/l) and renal failure (creatinine > 265 μmol/l) accounted for 5% and 3% of the cases respectively. In contrast to this 32% had features of liver dysfunction, however it appeared that haemolysis was the main contributing factor to the liver derangement. The most common infecting species was

Plasmodium falciparum, alone (91.3% of the patients) or part of a mixed infection with either *P.vivax* or *P.ovale* (3.8% of patients). More than 88% of the infections were contracted in other African, mainly southern African countries, the most important of which was neighbouring Mozambique (58%). About 11% were contracted in endemic areas of South Africa i.e Northern Province, Mpumalanga and Kwazulu Natal. Twelve percent of cases (24/203) gave a history of previous malaria. The underlying immune status of these patients was analyzed using the Indirect Fluorescence Antibody Test (IFAT) and compared with the total study group. The test did not reveal any striking differences between the two groups. The previously exposed patients however did demonstrate a much lower parasitaemia, with 71% (17/24) of cases presenting with a parasite density ≤ 1%. These results may indicate the ability of these patients to clear their parasitaemias earlier due to previous sensitization, with the subsequent establishment of a low grade chronic infection.

Seven of 88 women admitted had a documented pregnancy at the time of diagnosis. Foetal death was recorded in 5/7 cases which confirms the poor prognosis in pregnancy associated malaria infection, reported by other authors.

Fifteen patients (13 adults and 2 children) required admission into the intensive care unit. Indications included high parasite loads, > 5% (67%) and renal failure, creatinine > 265 μmol/l (33%).

Standard chemotherapy was administered to all the patients with the most frequently used being quinine (94% of cases), alone or in combination with other drugs. The use of prophylactic agents for the prevention of malaria was restricted to twelve patients (8%),

with the majority of individuals being ignorant about malaria and therefore being unaware that any medication along with other preventative measures, were necessary prior to entering , and while staying in an endemic region. It was also apparent that the correct dosage was not adhered to as none of the patients completed their antimalarial course after returning from the malaria area. The most commonly used prophylactic drugs were chloroquine, alone or in combination with proguanil and pyrimethamine plus dapsone (Maloprim). The latter is no longer recommended routinely as a prophylactic agent.

Following univariate analysis using Fisher's exact test and the student t-test, and a multivariate analysis (using a logistic regression model), hyperparasitaemia ($p=0.0070$) and renal failure ($p=0.0016$) were identified as significant predictors of poor outcome. Significant differences were also demonstrated in the mean WCC and the mean HB levels between the survivors versus the patients that died, indicating that a significantly elevated WCC and an anaemia at presentation, may be important risk factors towards the establishment of severe/complicated infection. The overall mortality rate was 3%.

Climatic data (which was limited to the Johannesburg area), together with evidence that the malaria bearing *Anopheles* vector does not exist in Gauteng suggests that the conditions in the city may not be suitable for local transmission of malaria during the summer. It therefore appears that all efforts need to be channeled into the education of our travelers who visit malaria endemic regions and upon returning succumb to 'imported' malaria. Perhaps the education of the traveler alone is not sufficient, and medical personnel who diagnose the condition and who prescribe prophylactic agents

need to revise their knowledge of this life threatening infection, so that their advice and drug therapies are optimal and effective. Lastly the pharmaceutical companies that manufacture these drugs might be persuaded to make their products more affordable for those individuals who are most at risk. We need to utilize our limited options to the fullest until such a time as the ultimate challenge is realised - an effective malaria vaccine.

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LIST OF ABBREVIATIONS

ALKPHOS	Alkaline phosphatase
ALT	Alanine transaminase
AST	Aspartate transaminase
ATP	Adenosine tri phosphate
CAT	Catalase
CNS	Central Nervous System
CREAT	Creatinine
CS	Circumsporozoite
CQ	Chloroquine
CQR	Chloroquine Resistant
CQS	Chloroquine Sensitive
CVS	Cardiovascular System
DALYs	Disability adjusted life years
DB	Direct bilirubin
DDT	Dichloro-dipheryl-trichloroethane
DIC	Disseminated Intravascular Coagulation
DNA	Deoxyribonucleic acid
EBA-175	Erythrocyte Binding Antigen - 175
EDTA	Ethylenediamine tetracetate
FBC	Full Blood Count
FITC	Fluoroscein Isothiocyanate
FP IX	Ferritoprotoporphyrin IX
γ-GT	γ-Glutamyltransferase
GLUC	Glucose
G6PD	Glucose-6-Phosphate Dehydrogenase
Hb/HB	Haemoglobin
HBcAg	Hepatitis B core antigen
HBsAg	Hepatitis B surface antigen
HCT	Haematocrit
HLA	Human Leucocyte Antigen
IB	Indirect bilirubin
ICAM-1	Intracellular Adhesion Molecule-1
ICU	Intensive Care Unit
IFAT	Indirect Fluorescence Antibody Test
IFN γ	Interferon γ (gamma)
Ig	Immunoglobulin
IL	Interleukin

LOC	Loss of consciousness
LSA-1	Liver Stage-specific Antigen -1
LT α	Lymphotoxin α (alpha)
Max	Maximum
MAPs	Multiple antigen peptide system
MCH	Mean Cell Haemoglobin
MCHC	Mean Cell Haemoglobin Concentration
MCV	Mean Cell Volume
MEPs	Multiple epitope peptide system
MESA	Mature parasite infected Erythrocyte Surface Antigen
Min	Minimum
MSP/A-1	Merozoite Surface Protein/Antigen - 1
NO	Nitric Oxide
NOS	Nitric Oxide Synthase
PCR	Polymerase chain reaction
PCT	Parasite clearance time
pers.comm.	personal communication
P*f*EMP-1	*Plasmodium falciparum* Erythrocyte Membrane Protein -1
P*f*HRP-1	*Plasmodium falciparum* Histidine Rich Protein-1
PLTs	Platelets
PMMSA	*Plasmodium* Major Merozoite Surface Antigen
PRBC	Parasitised red blood cell
PUO	Pyrexia of unknown origin
PVM	Parasitophorous vacuole membrane
RBC	red blood cell
rDNA	ribosomal deoxyribonucleic acid
RESA	Ring infected Erythrocyte Surface Antigens
RNA	Ribonucleic acid
rRNA	ribosomal ribonucleic acid
SEA	South East Asian
SERA	Serine Repeat Antigen
SOP	Standard operating procedure
SSP-2	Sporozoite Surface Protein - 2
STD	Standard deviation
TB	Total bilirubin
TNF α	Tumour Necrosis Factor α (alpha)
TP	Total protein
TSP	Thrombospondin
U+E	Urea and electrolytes
VCAM-1	Vascular Cell Adhesion Molecule -1
WCC	White cell count

The Ballad of the *Plasmodium*

Plasmodium has a lot in store
And works in stages by the score.
Anopheles that probes your skin
Pumps many sporozoites in,
They lose no time, move into liver
And settle down before you shiver,
Some hypnozoites go to sleep
A late relapse intending to keep.
But others grow,divide like mad
And move from liver into blood.
In red cells do their very worst
Expanding to make each cell burst
As merozoites they are vexed
By being greatly undersexed.
Their life is tedious,rather stale
Without a female and a male
This truth they soon will realize
And change assuming larger size
So now, when a mosquito bites
It must suck up gametocytes
And microgametes being sucked
Can now perform the amorous act
When in the stomach of the gnat
They find a macrogamete fat
Their love's great feat is now complete
As female ookinete
Then losing all her self-control
She goes through the insect's stomach wall.
And there encysted feels more able
To raise a new *Plasmodium* stable
When thousand sporozoites fit
Will prove that she has done her bit
To salivary glands they wend
And start again; there is no end.
The moral of this age-old story
Is that we can't aspire to glory
Until our principal objective
Will make control much more effective
And faced with chloroquine resistance
We must depend on more assistance
And find new medicament
To solve our great predicament
A compound that in all event
Is active cheap,polyvalent.
Perhaps we need a proper vaccine
With antigens that science mucks in
And adjuvants that will not itch
And antibody God knows which.
Or to develop other means
To fight those vicious little fiends
That no one who is from Mankind Could e'er defend, if sound of mind.

Leonard Bruce-Chwatt

1.0 INTRODUCTION

In 1994 and 1995 the total number of malaria cases reported in South Africa were over 10 000 and 9 000 respectively (Sharp and le Sueur ;1996). Despite the many efforts to eradicate this disease worldwide, the number of cases detected every year continue to increase. This can be attributed to heavy seasonal rainfalls, resistance of the mosquitoes to insecticides, and resistance of the parasite to the standard chemotherapeutic agents used, eg. Chloroquine . Another postulate is that, the populations that are being exposed to malaria may have now lost a previously semi-immune state to the parasitic infections.

Malaria remains the most important tropical parasitic disease , with major social and economic implications in developing countries. The document that follows discusses the incidence of malaria at the Chris Hani/Baragwanath Hospital in Gauteng, South Africa .

1.1 HISTORICAL BACKGROUND

Earliest records of malaria date back to the 5 th century B.C. when Hippocrates differentiated types of fevers and coincidentally described the clinical picture of malaria and some of its complications.

In southern Africa malaria appears to have been around from the time of the explorers, it is also probable that the disease may have originated on the continent (Gilles,1993).

The important milestones in the history of malaria (adapted from Gilles H.M. in Bruce-Chwatt's Essential Malariology,1993, p.4) are listed below:

Milestones in the History of Malaria

30 Million years BC	**Fossil mosquitoes found in geological strata.**
500 BC	**Hippocrates describes the clinical picture of malaria. He observes an association between the emergence of intermittent fevers, seasons of the year, and swampy / stagnant watery environments.**
1600 AD	**Jesuit missionary records the use of the *fever tree bark* (Jesuit's Powder) - Cinchona tree, for the treatment of certain fevers (agues).**
1820	**The alkaloids quinine and cinchonine were isolated from the bark of the Cinchona tree, by two frenchmen.**
1880	**A French army surgeon in Algeria, first saw and described malaria parasites in the RBC of human blood.**
1886	***P.vivax* and *P.malariae* were described.**
1889 - 1890	***P. falciparum* was described.**
1891	**The Russian Romanowsky developed a new method for staining malaria parasites in the blood.**
1894	**Manson puts forward his theory that malaria is transmitted from one person to another via a mosquito.**
1898 - 1899	**The Italians Grassi et al described the cycle of human malaria in the Anopheles mosquito.**
1899	**The first antilarval programmes were started in Sierra Leone.**
1901	**The existence of a pre - erythrocytic phase in the life cycle of the malaria parasite was hypothesised.**
1922	***P. ovale* was described.**
1934	**Chloroquine was developed in Germany.**
1935 - 1939	**First application of insecticidal measures (including DDT) for the control of malaria.**
1944	**Proguanil was developed in England.**

1949	**Shortt et al described the pre - erythrocytic stage of *P. falciparum* in the human liver.**
1952	**Pyrimethamine and primaquine were developed in the USA.**
1957	**The WHO launches a global campaign for the control of malaria.**
1961 -1965	**Emergence of chloroquine resistant strains of *P. falciparum.***
1971 -1975	**Introduction of mefloquine into malarial treatment.**
1978	**Riekmann introduces a microtest for the detection of chloroquine resistance in *P. falciparum.***
1979 - 1982	**Development of the quinghaosu antimalarials.**
1985 - 1986	**WHO recommends that malaria control should be developed as an integral part of national primary health care systems.**
1989	**Halofantrine was introduced.**
1990 -	**Artenusate and artemether are used in clinical trials**
	PCR used to detect malaria; may be used to provide information regarding phenotypic characteristics of various *Plasmodium* strains.
	A simple rapid manual dipstick test (PARASIGHT - F) to detect *P. falciparum* infections, is described.
	Patarroyo's field trials of the S Pf 66 vaccine show it to be safe. Protective efficacy against *P. falciparum*, however was found not to be optimal in areas of high malarial endemicity.

1.2 EPIDEMIOLOGY

Of the total world population of +/- 5 000 million people, 41% (+/- 2 200 million people) live in malarial areas (Murphy and Oldfield, 1996; WHO Bulletin, 1992).

Figure 1 shows the worldwide distribution of malaria. Countries in tropical Africa account for >80% of the +/- 120 million clinical cases reported annually, and for >90% of the nearly 300 million parasite carriers. In Africa alone, malaria displaces pneumonia as the first ranked cause of lost DALYs - disability-adjusted life-years (World Bank. World Development Report ,1993).

The disease can affect any age, however children and pregnant women are often at a greater risk. A current estimate of childhood deaths in Africa is 800.000/year, killing one out of twenty children before they reach 5 years of age (Murphy et al, 1996). Malaria may also be imported into non-malarious areas by travellers returning from endemic regions, and occasionally within mosquitoes . Here the mosquito (*Anopheles* species) survives trips on airplanes, boats, and minibus taxis', giving rise to airport/taxi - rank malaria.(Subramanian et al, 1992; Wyler, 1993; Layton et al, 1995).

Increased population migration (of parasite carriers) as a result of factors such as family ties, war, famine, and job seeking behaviour also facilitates the transmission of disease between countries (le Sueur et al, 1996; Sharp and le Sueur, 1996). Very infrequently, infection may be acquired congenitally from an infected mother, or through blood transfusion (Bruce-Chwatt, 1982; Babinet et al, 1991; Norse, 1993; Miller and Telfor, 1996; Lane and Edwards, 1997).

Reports of nosocomial transmission have also been documented recently (Abulrahi et al, 1997).

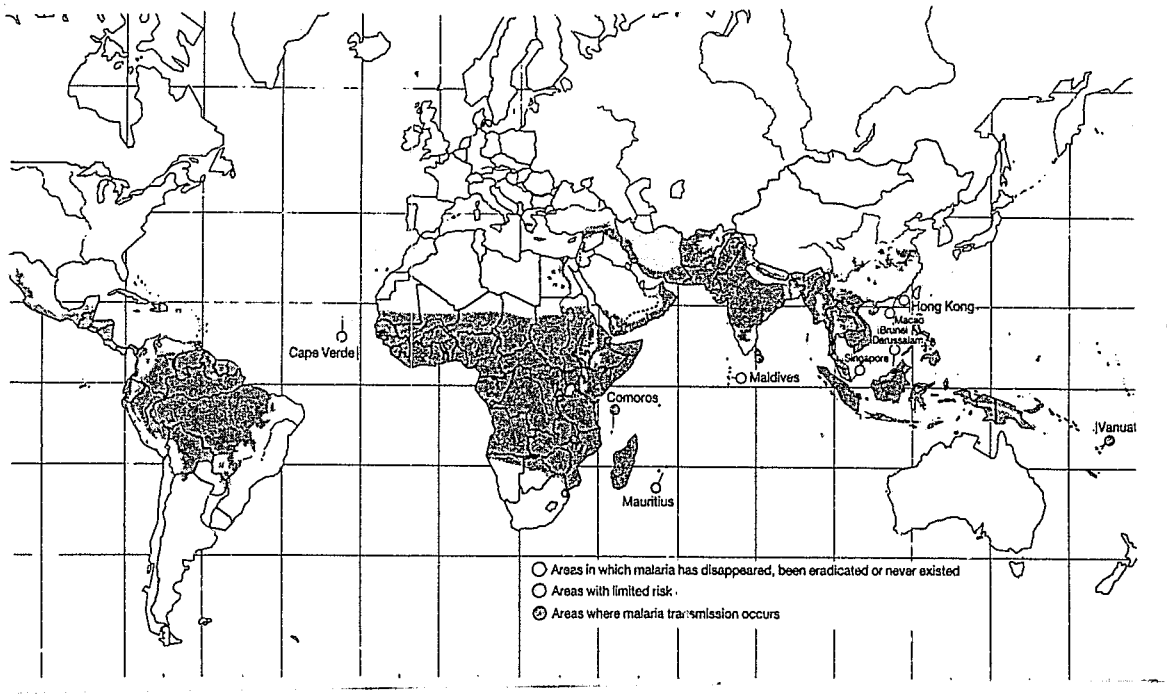


Figure 1: The Worldwide Distribution of Malaria

WHO Bulletin, 1992

The degree of malarial transmission can vary greatly from one region of a country to another, where variables such as altitude, terrain and temperature influence the mosquito vector. In other areas transmission of malaria is seasonal due to fluctuations in rainfall, as this can influence mosquito breeding. Recent climatological changes like global warming and the El Nino effect are thought to contribute to the increasing malarial problem by providing circumstances favourable to malaria transmission (Lindsey and Birley, 1996; Olliaro et al, 1996). With increasing population in areas at high risk for malaria, and with malaria occurring in previously transmission-free regions due to the human impact on, and interaction with the environment the predicted effect of

this disease in terms of numbers of infected individuals could increase dramatically during the next decade.

In South Africa malaria is found in the lower altitude areas of the Northern Province, Mpumalanga, and Kwazulu-Natal (Figure 2), where it has had devastating effects on the communities, tourism, agricultural and industrial development in these provinces; especially the Kwazulu Natal and Mpumalanga regions. In 1932 22 000 malaria related deaths were reported in Kwazulu Natal, in one of the worst epidemics to be described in this country (Sharp and le Sueur, 1996). High risk areas include all border regions, indicating the importance of imported malaria.

Imported cases of malaria have been reported from all countries of southern Africa, with the majority appearing to originate from Mozambique. Sharp and le Sueur (1996) in a recent South African Medical Journal publication highlighted the importance of viewing malaria as a regional problem and not just a country-specific one.

1.3 BIOLOGY

The infection is caused by four species of protozoal parasites of the genus *Plasmodium* (Table 1) . This genus has been defined on the basis of a) the type of asexual multiplication (schizogony), by division occurring in cells other than the erythrocytes of the vertebrate host (exo-erythrocytic schizogony) and b) the mosquito hosts , which are various species of Diptera. The zoological classification of *Plasmodia* is complex and there appears to be a lot of difference of opinion regarding the position of parasite species within groups and sub-genera (Service, 1993) .

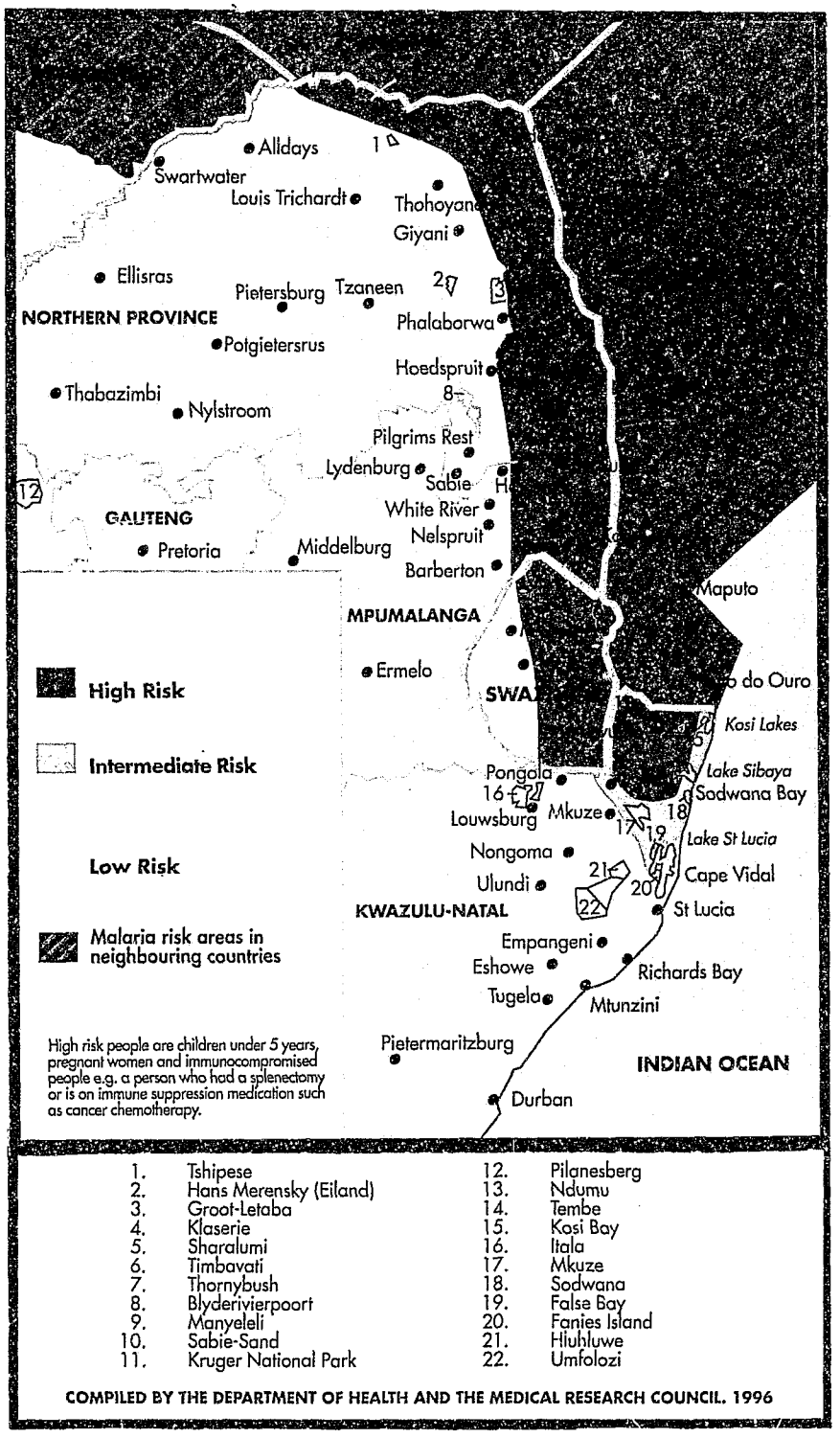


Figure 2: **Malaria Risk Areas in South Africa**

Different *Plasmodium* species predominate in different geographic regions. For example *Plasmodium falciparum* accounts for most of the clinically significant infections in Africa, and for over a third of infections in the rest of the world. *Plasmodium vivax* remains the major species transmitted in parts of India, and is virtually absent in Africa (as most Africans lack the Duffy blood group antigens that define susceptibility to this parasitic species). *P.malariae* has a patchy distribution in China, has also been described in East and West Africa, and in some parts of South America. *P.ovale* has been associated with mixed infections in Tropical Africa.

Table 1: The classification of *Plasmodia* infecting primates and other mammals

Genus: *Plasmodium*		
Sub-genus : *Plasmodium*		
Group: Vivax	Species:	*P. vivax*,P.cynomolgi P. eylesi, P. pitheci P. gonderi, P.hylobati P. jeffeyi, P. schwetzi Psimium,P.sylvaticum P. youngi*
Group: Ovale	Species:	*P. ovale*, P. fieldi P. simiovale*
Group: Malariae	Species:	*P. malariae*,P.inui P.brazilianum*
Group: Uncertain	Species:	*P. catneyi, P. fragile P. knowlesi*
Sub-genus: *Laverania*	Species:	*P. falciparum*, P. reichenowi*
Sub-genus: *Vincheia*	Species:	large number of species infecting,lemurs,rodents bats and other animals.

* signifies *Plasmodia* infecting human

Service, 1993

All four *Plasmodium* species are transmitted by a mosquito vector of the genus *Anopheles.* These insects have a few important characteristics that may be relevant for the transmission of infection:

- Feed from dusk to dawn.
- Only the female mosquito feeds on vertebrates (blood is needed for egg production).
- The mosquitoes survive long enough (9-22 days) after feeding to transmit the infection (this is dependent on temperature, predators [bats,birds], and vector controlling methods [insecticides]).
- Elevations above 6000 feet are not conducive to transmission.
- Mosquitoes are resilient enough to survive travel in motor vehicles and airplanes,to transmit infection.

1.3.1 Life Cycle of the *Plasmodium*

Infection is initiated when the female anopheline injects saliva and malaria sporozoites into the skin of the victim during feeding (blood meal). The sporozoites are rapidly cleared by the liver,and within minutes they penetrate hepatocytes and transform into hepatic exo-erythrocytic forms or tissue schizonts. After ~ 1 week each tissue schizont has produced thousands of daughter merozoites which are released from the liver into the circulation. The intrahepatic development results in no symptoms or a mild derangement of liver function. In patients with *P.vivax* and *P. ovale* infections, the exo-erythrocytic forms can remain dormant (hypnozoites) for weeks or months after the initial malarial attack. These hypnozoites can later become activated and continue their development into merozoites, resulting in relapses of disease which often occur repeatedly.

Once in the circulation the merozoites invade erythrocytes and start the intra-erythrocytic cycle, forming ring (early) trophozoites. The intracellular parasites feed on the haemoglobin, and through the process of schizogony (asexual replication) give rise to mature blood schizonts (up to 32 daughter merozoites per schizont). This development occurs over ~ 48 hours (longer in *P. malariae* ~ 72 hours) . Schizogony progresses from the initial ring form (one or two characteristic purplish chromatin dots attached to a bluish, ring shaped cytoplasm) to late trophozoites (larger and lacier - the cytoplasm has an amoeboid appearance and the chromatin is less dense) to the early blood schizonts (with several merozoites whose individual nuclei may be seen) and finally to the mature schizont. Lysis of the erythrocyte releases the merozoites which then invade other red cells. After about two to three cycles parasitaemias become detectable on microscopy.

The period from inoculation of the parasite by the mosquito to the appearance of parasitaemia *(prepatent period)* is usually 9 - 13 days, but longer periods are possible. The time from inoculation to the first symptoms of disease is the *incubation pericd* and its length usually depends upon the patient's immune status. Normally the incubation period is ~ 11-15 days. In non-immune patients symptoms of the infection may develop prior to the detection of a parasitaemia. The other extreme occurs in areas with heavy malarial transmission where patients have developed immunity to the infection; here parasitaemias may be detected in the absence of any symptoms.

After several cycles some of the late trophozoites, within the erythrocytes, develop into sex cells - gametocytes.. The mature gametocytes have different forms in different

species of *Plasmodia.* In *P. falciparum* for example they are usually crescent-shaped/banana-shaped while in the other species they appear round. Gametocytes can remain in the circulation for long periods of time after the asexual intraerythrocytic stages are cleared. They cause no clinical symptoms and require no treatment, but these forms are infectious to the *Anopheles* mosquito. If male and female gametocytes are taken up by the anopheline during a blood meal, they can mate and form oocysts (this development usually takes place outside the wall of the midgut). The oocyst gradually increases in size and starts to subdivide eventually leading to the formation of elongated spindle -shaped sporozoites. The sporozoites emerge from the oocysts, invade the body cavity of the mosquito and migrate to the salivary glands, where they remain until the next blood meal. The mosquito cycle (Figure 3) is completed within 2 weeks to 25 days.

Since *P.falciparum* is the most important human parasite,from the clinical point of view, I have confined the rest of my discussion mainly to this species.

1. 3.1.1 The parasite in the vertebrate host - invasion of the erythrocyte

(a) *Initial interaction*

The merozoite is the form of the malaria parasite that attaches to and enters the erythrocyte. It is a lemon shaped structure ~ 2 μm in length, covered by a coat of protein bristles (~ 195 kDa major merozoite surface proteins - PMMSA; MSP-1) which are loosely rooted in the membrane. It is these sites which are thought to be involved in the initial interaction with the host cell. As the merozoite collides with the red blood cell, a weak (electrostatic) surface interaction occurs. Figure 4 shows the interaction between the merozoite and the red blood cell.

(b) *Reorientation*

The randomly adhering merozoite then orientates itself such that its apical end is in apposition with the red cell membrane.The flattened conical - shaped apical region contains the rhoptries, a pair of club shaped,membrane- bound and electron dense internal organelles, and the tubular structures known as micronemes.

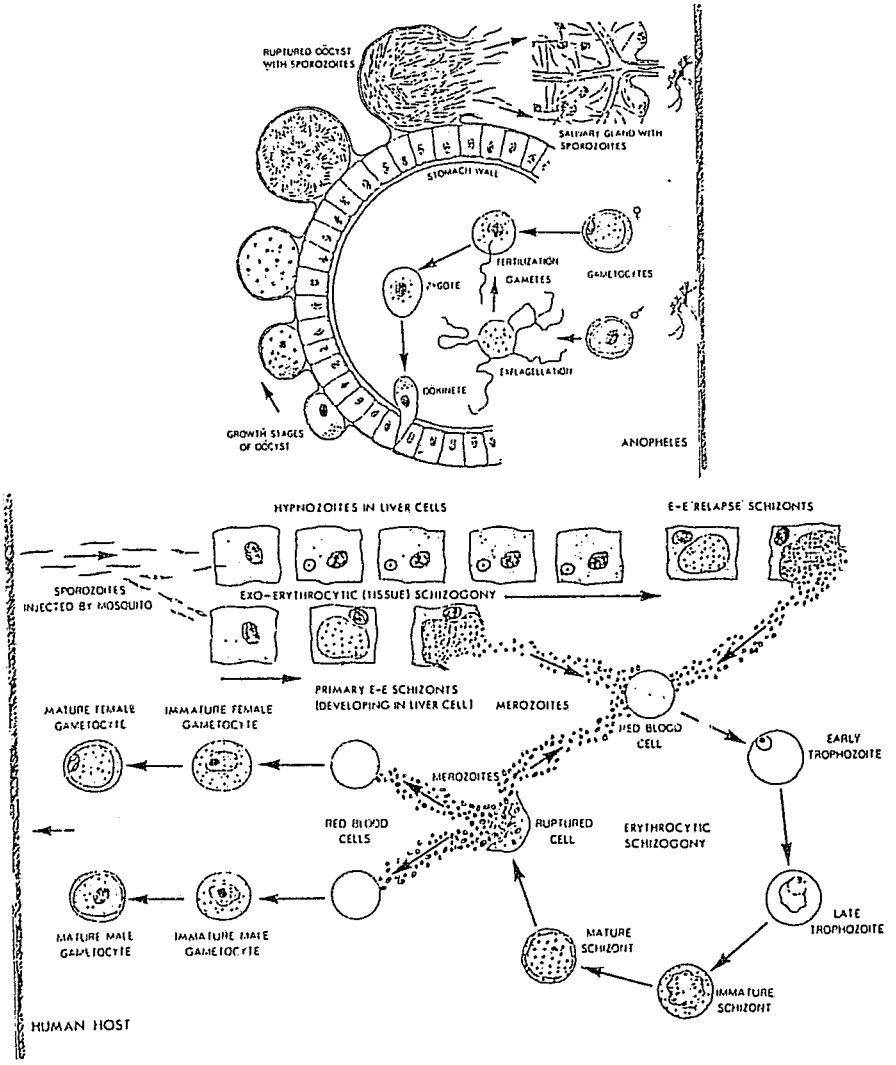


Figure 3: Developmental stages of the Malaria Parasite in *Anopheles* mosquitoes and in the human host.

In: Gilles H.M., Warrell D.A., editors. *Bruce-Chwatt's Essential Malariology.* Third Edition. London: Edward Arnold.

Identification of the molecules on the red cell surface to which the merozoites bind has been the subject of intense research (Pasvol, Clough and Carlsson, 1992). The sialic acid rich glycophorins especially Glycophorin A and Glycophorin B have been shown to play a major role in the invasion of the erythrocyte by malaria parasites, especially *P.falciparum*. Other antigens expressed on the red cell surface have been implicated in malaria invasion e.g the Duffy antigens which are important in *P. vivax* infection.

Efficient and successful invasion by the merozoite requires the release of erythrocyte binding proteins . EBA - 175, erythrocyte binding antigen-175 , is a 175 kDa molecule which has been localised to the micronemes. This protein binds to the sialic acid linked tetrasaccharides of the glycophorins . A specialised conserved region , EBA peptide 4 has been identified within this molecule. It is postulated that this region (44 amino acid residues in length) is particularly important for red cell entry as antibodies manufactured to this peptide blocked the binding of the parent molecule to the erythrocyte and inhibited invasion by the merozoite (Sim , 1995).

(c) *Internalisation*

After attachment and reorientation of the merozoite occurs, an invagination develops opposite the apex of the parasite. This invagination deepens gradually so that eventually the merozoite slips into the erythrocyte. The point of contact with the host cell membrane is defined by an electron-dense "junction" . This "junction" contains a high concentration of intramembrane molecules e.g glycophorins and band 3 .The area however that is bound by this annular junction is devoid of the red cell intramembrane

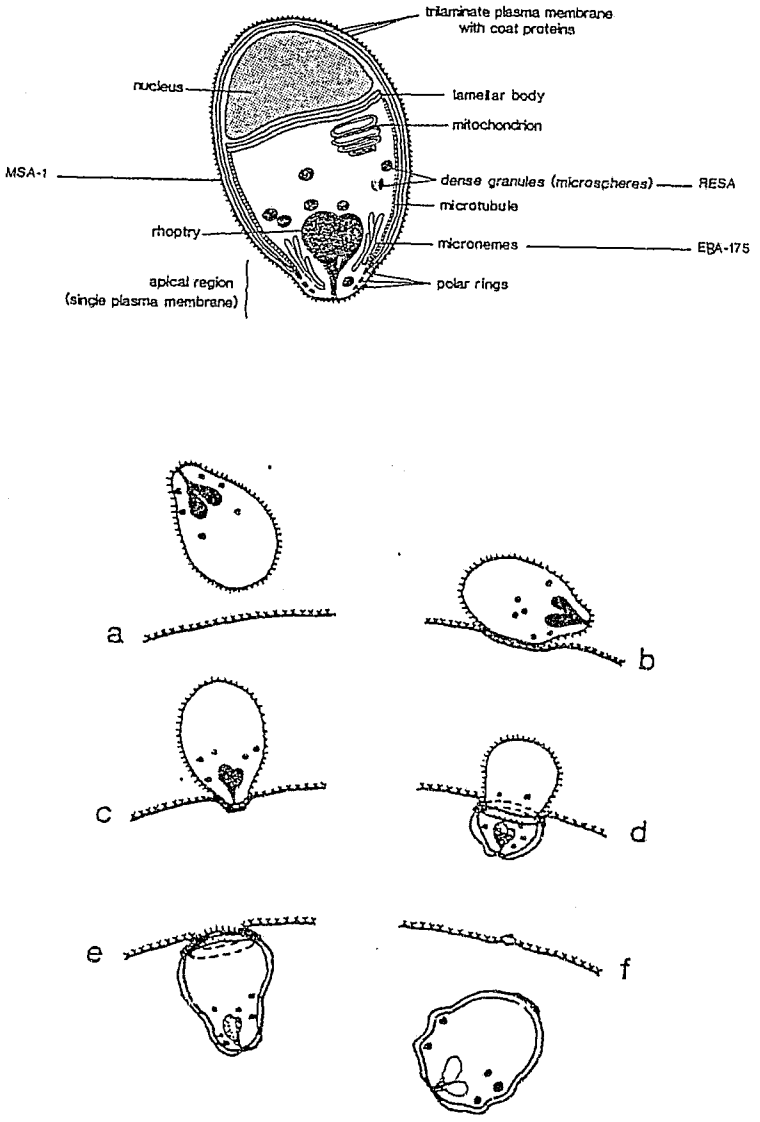


Figure 4: **Schematic view of the malaria merozoite and its interaction with the host red blood cell surface: (a-b, initial interaction; c, orientation; d-f, internalisation)**

Compiled from : Pasvol et al, 1992; Pasvol et al, 1993

components. The mechanism which results in the outward diffusion of these molecules to create this 'bare zone' is not clear. The contraction of the cytoskeletal components appears to be coupled to ankyrin and/or protein 4.1 and seems to be an ATP-dependent step. Simultaneously the micronemes and the rhoptries discharge their contents into the membrane resulting in a local expansion of the membrane and eventually the formation of the parasitophorous vacuole membrane (PVM) (Sam-Yellowe, 1996).

The merozoite slowly moves into the invagination and developing vacuole through the "junction" (which appears to "slide" backwards along the surface of the parasite); during this process there is loss of the outer two membranes, and the parasite starts to assume a rounded form. Once the parasite is engulfed the dense granules which are also distributed at the apical region are released. It is thought that the proteins contained by the granules e.g Pf155/RESA (ring-infected erythrocyte surface antigen of 155kDa) may play a role in enabling the trailing end of the invaginated red cell membrane to fuse, forming a plug thereby enclosing the merozoite completely within the parasitophorous vacuole (Foley and Tilley,1995). To date there is a controversy as to the origin of the PVM. There are arguments that support a host cell derivation as well as possible that the vacuole arises from elements contributed by both the parasite and the host cell. Although the red cell is capable of endocytosis the structured way that the merozoite enters the host cell suggests a specific mechanism of invasion that is determined by both parasite and red cell factors:

(i) *Metabolic pathways*

Researchers have demonstrated that invasion appears to be dependent on the presence inside the red blood cell of calcium (Ca^{2+}) , magnesium (Mg^{2+}) and adenosine triphosphate (ATP), which is particularly important by maintaining the assymetric

distribution of the phospholipid bilayer and for its role in the phosphorylation/dephosphorylation of the cytoskeletal components of the RBC membrane (Wasserman, 1990; Wilson, 1990; Gratzer and Dluzewski, 1993; Haynes, 1993).

(ii) *Proteases and Proteolysis*

A number of parasite proteases have been identified ,which are involved in numerous physiological events like the digestion of haemoglobin (this will be discussed in detail later in the text), or the invasion of the RBC and probably in the release of the parasite from the host cell (Schrevel et al, 1990; Gratser and Dluzewski, 1993; Haynes, 1993). The infected RBC contain numerous compartments with different pH ranges which result in varying protease activities.

(iii) *Membrane rigidity and abnormal red cells*

It has been well documented that certain individuals are less susceptible to malaria infection, and some of this resistance is based on genetic features that affect several stages of the intraerythrocytic cycle of the *plasmodia* (Hill, 1992; Yathavong and Wilairat, 1993; Oo et al, 1995). Certain haemoglobinopathies like HbS, HbE, alpha and beta thalassaemia , and enzymeopathies like G6PD deficiency are protective to the carriers because they inhibit the intraerythrocytic growth period. A number of mechanisms have been suggested for the reduced survival of the parasite under these conditions (Nagel, 1990), these include metabolic disturbances in the red blood cells of the 'sickling disorders'; oxidant damage and increased susceptibility for cell mediated removal from the circulation. The abnormal haemoglobins including the presence of persistently high HbF, are thought to be a poor substrate for the parasite proteases.

Another red cell factor that determines whether invasion by the parasite will be successful is the mechanical rigidity of the RBC membrane (Gratzer and Dluzewski, 1993; Haynes, 1993; Pasvol et al, 1993). Disorders like Southeast Asian ovalocytosis, (an abnormality within band 3), Hereditary Elliptocytosis (deficiency of protein 4.1) and Hereditary Pyropoikilocytosis (abnormalities in the spectrin molecules) result in disruption of the cytoskeletal network (Figure 5) and lead to decreases in membrane elasticity, and reduced susceptibility for invasion (Nurse, Coetzer and Palek, 1992).

(iv) *Age of host cell*

It is thought that as the rbc ages it becomes less susceptible to invasion, hence reticulocytes are a favoured target. This theory may not to be entirely correct as the type of cell that becomes invaded may be species specific.

There appear to be properties inherent to the red cell e.g small differences in the physical properties of the membrane, like the elimination of elements from the glycocalyx (including receptors), and increasing cell density which may all be a function of age ; as well as parasite specific factors which may influence the type of cell that is infected. (Gratzer and Dluzewski.,1993).

(v) *Merozoite motility*

Several motor proteins have been identified which are thought to be involved in propelling the merozoite into the RBC (Bannister and Dluzewski, 1990; Gratzer and Dluzewski, 1993). Using an immunofluorescence technique actin has been found in a soluble form in the cytoplasm of the merozoite, and membrane bound in the form of filaments.

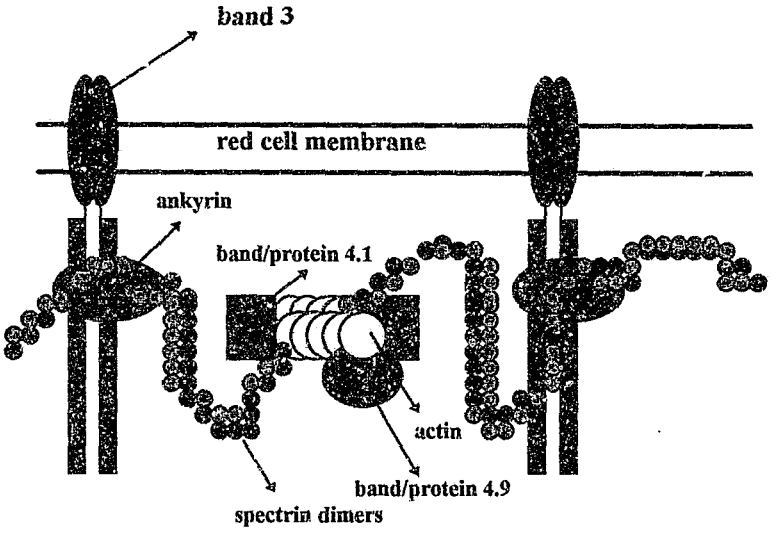


Figure 5: A model of the cytoskeletal organisation of the erythrocyte.
Reproduced from Foley and Tilley, 1995

1.3.1.2 The parasite in the vertebrate host - changes in the parasitised red blood cell

Even after invasion of the RBC, the parasite continues to modify its host.

(a) *The membrane of the parasitised cell*

(i) *Transport functions of the parasitised erythrocyte membrane.*

Several differences have been identified in the parasitised cell membrane, which are illustrated in Figure 6. These include various permeation pathways, membranous vesicles and tubules which have been created between the PVM and the RBC membrane to facilitate increased movement of nutrients and substrates in and out of the cell (Barnwell, 1990; Atkinson and Aikawa, 1990; Schrevel et al, 1990; Haynes, 1993).

(ii) *Lipid changes in the parasitised membrane.*

The intraerythrocytic development of the *Plasmodium* within the mature erythrocyte is associated with active membrane biogenesis of neutral lipids and phospholipids, to ensure the increase in the size of the parasite and the parasitophorous vacuolar membranes. Notably the phospholipid composition appears to differ markedly from that of the original host cell membrane (Vial et al, 1990; Pasvol et al, 1992). The mature uninfected erythrocyte is essentially incapable of any lipid biosynthetic activity. The parasite accumulates lipids and fatty acids from the serum , and even uses the red cell membrane as substrate, to synthesise its own lipids.

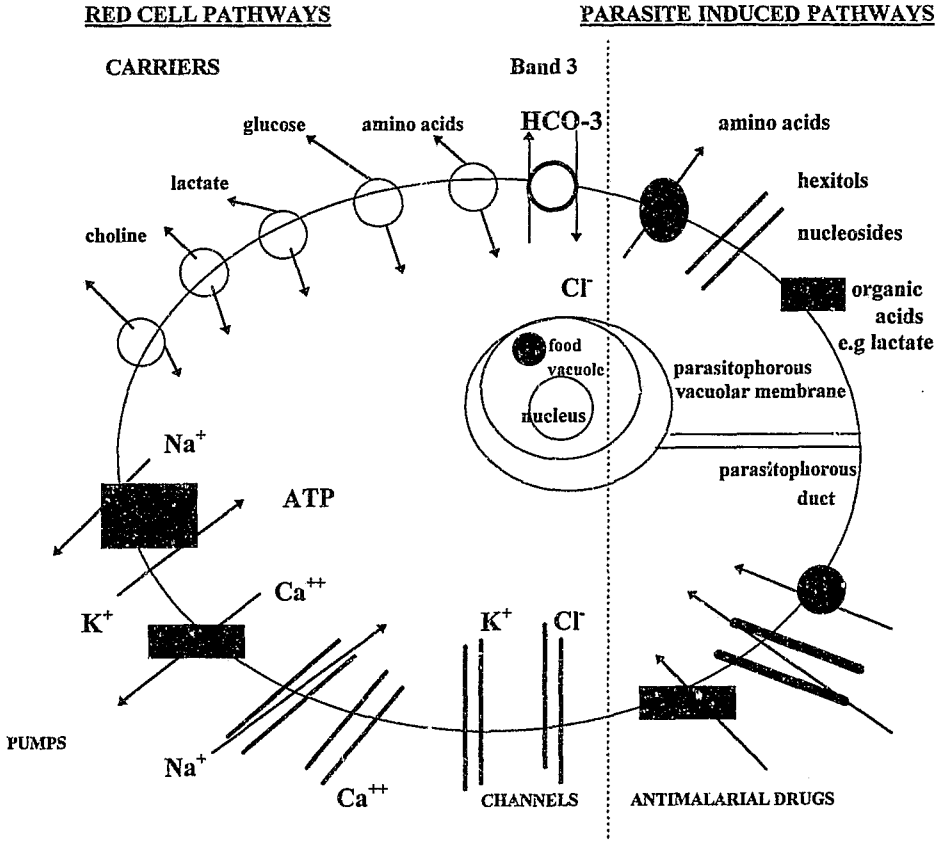


Figure 6: **Schematic representation of permeation pathways in the host membrane of malaria infected cells.**

(iii) *Cytoadherence and Rosetting*

Once the red cell is infected with mature parasites it gains the ability to 'bind' or adhere to : 1) endothelial cells, mainly in the capillaries of key organs like the brain, heart, liver, lung and kidney ,and no longer circulates in the peripheral blood (this phenomenon is known as sequestration) ; 2) other red blood cells to form rosettes ; and 3) different types of cells in the circulation, such as monocytes, neutrophils and lymphocytes. Cooke et al (1994) suggest that the above interactions may represent a strategy developed by the parasite to avoid destruction in the reticuloendothelial system . It is these interactions, nevertheless, that are thought to be responsible for the severe pathology of falciparum malaria (this will be discussed further in the clinical section).

The mechanism of cytoadhesion is multifactorial. With the aid of electronmicroscopy a number of regular, symmetrically arranged "knobs" (Figure 7) have been identified on the surface of the parasitised red blood cell (PRBC). (Pasvol et al, 1992; Gratzer and Dluzewski, 1993). These knobs are cone shaped and comprise of intramembrane particles, which form clusters that eventually become a raised densely packed central zone. Immunofluorescent labelling suggests an elevated concentration of band 3, glycophorin, and spectrin in these regions. (Pasvol et al, 1992; Gratzer and Dluzewski, 1993; Haynes, 1993; Atkinson and Aikawa, 1990). Other ultrastructural alterations have also been described: caveolae and caveola-vesicle complexes (in non-falciparum species), and cytoplasmic clefts (Atkinson and Aikawa, 1990). These will not be discussed further.

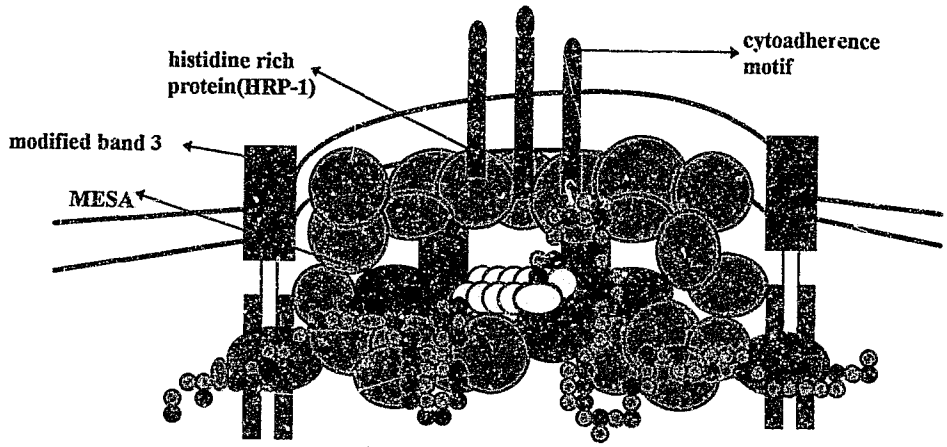


Figure 7: **Cytoskeletal organisation of an erythrocyte infected with a mature stage 'knobby' parasite [MESA: mature parasite infected erythrocyte surface antigen]; please refer also to figure 5, page 18**

Reproduced from Foley and Tilley, 1995

Several proteins/antigens have been associated with the knob-associated electron-dense material in *P.falciparum* (Foley and Tille,1995). Table 2 summarises the proteins that interact with the erythrocyte cytoskeleton.

Cytoadherence to the vascular endothelium and rosetting of *Plasmodium falciparum* - infected red blood cells appears to be a major factor in the pathogenesis of severe malaria. Several molecules have been shown to be potential receptors on the endothelial cells and erythrocytes for infected red blood cells (Roberts, Sherwood and Spitalnick et al, 1985; Barnwell, Asch and Nachman et al, 1989; Berendt, Ferguson and Newbold, 1990; Ockenhouse, Tegoshi and Maeno et al, 1992; Cooke et al, 1994; Wahlgren et al, 1994; Cooke and Coppel, 1995; Land et al, 1995; Gardner et al, 1996).

Table 2: *Plasmodium falciparum* proteins which interact with the erythrocyte cytoskeleton

Protein[a]	Apparent molecular mass (kDa)	Stage, Location, Properties	Host Receptors	References
RESA(P*f*155)	155	Ring stage; cytoplasmic surface; interacts with spectrin.		Foley and Tilley, 1995
MESA(P*f*EMP 2)	250-300	Mature stages; cytoplasmic surfaces; insoluble aggregate; interacts with spectrin may affect adhesion qualities of the cell.		Foley and Tilley, 1995
P*f*EMP 1 (*sequestrin*)	250-300	Mature stages; exposed on external surface; may traverse membrane and interact with the membrane skeleton; capable of antigenic diversity.	CD36 TSP ICAM-1 VCAM-1 E-Selectin	Baruch et al., 1996 Cooke et al., 1994 Cooke and Coppel, 1995 Gardner et al., 1996 Gratser and Dluzewski, 1993 Land et al., 1995 Nowak, 1995 Pasvol et al., 1992
MSA 1 (MSP 1)	190	Mature stages; merozoite surface and parasitophorous vacuole surface; interacts with *spectrin in vitro*.		
Rosettins	22-28	Mature stages; exposed on external surface; found exclusively in sequestering parasites and may play a role in cytoadherence and obstruction of the microvasculature.	CD36 ABO Ag	Wahlgren et al., 1994
Modified Band 3	-	Red blood cell cytoskeleton; contains regions (adhesins) that function in the attachment of the rbc to the endothelium.		Land et al., 1995
P*f*HRP 1	90	Ring stages; mature stages; attached to the cytoskeleton on the cytoplasmic face of the "knobs"; associated with spectrin and actin molecules; has a highly charged region which may participate in clustering and formation of the "knobs". Responsible for cytoadherence? A knobless phenotype has been described with altered cytoadhering properties.		Pologe and Ravetch, 1986

a Abbreviations: RESA, ring-infected erythrocyte surface antigen; MESA, mature parasite-infected erythrocyte surface antigen; HRP 1, histidine-rich protein 1; P*f*EMP1, *Plasmodium falciparum* erythrocyte membrane protein 1; MSA 1, merozoite surface antigen 1; TSP, Thrombospondin; ICAM-1, intracellular adhesion molecule 1; VCAM-1, vascular cell adhesion molecule 1; rbc, red blood cell.

- CD 36
- ICAM - 1 (intracellular adhesion molecule 1)
- TSP (thrombospondin)
- VCAM -1 (vascular cell adhesion molecule 1)
- E-Selectin
- ABO Blood Groups

The roles of the various receptors *in vivo* is not entirely clear, however a stepwise process to cellular adhesion, analogous to the way in which leucocytes achieve immobilization has been suggested. The expression of the receptors is often upregulated by inflammatory cytokines, for e.g TNFα (tumour necrosis factor α). With ICAM-1 readily detected on endothelial cells in the cerebral microvasculature, sequestration of PRBC occurs at these sites and this together with the mechanism of red cell rosetting, is an attractive model (Figure 8) for the pathogenesis of cerebral malaria (see section 1.4.1.1).

The type of blood group (A,B,AB,O) also appears to determine the risk of developing cerebral malaria. Hill et al have demonstrated a significant degree of protection against cerebral malaria amongst children with blood group O as compared to blood group A or B (Wahlgren et al.,1994). The protective effect conferred by blood group O may be related to the formation of smaller and weaker rosettes by blood group O red cells resulting in a less efficient "plugging" of the microvasculature of the brain.

Finally several investigators believe that the phopholipid composition of the PRBC membrane (i.e exposure of phosphatidylserine at the outer surface) may alter the RBC

surface charge thereby enhancing the adhesion of the infected erythrocyte to the endothelium and to other erythrocytes (Pasvol et al, 1992).

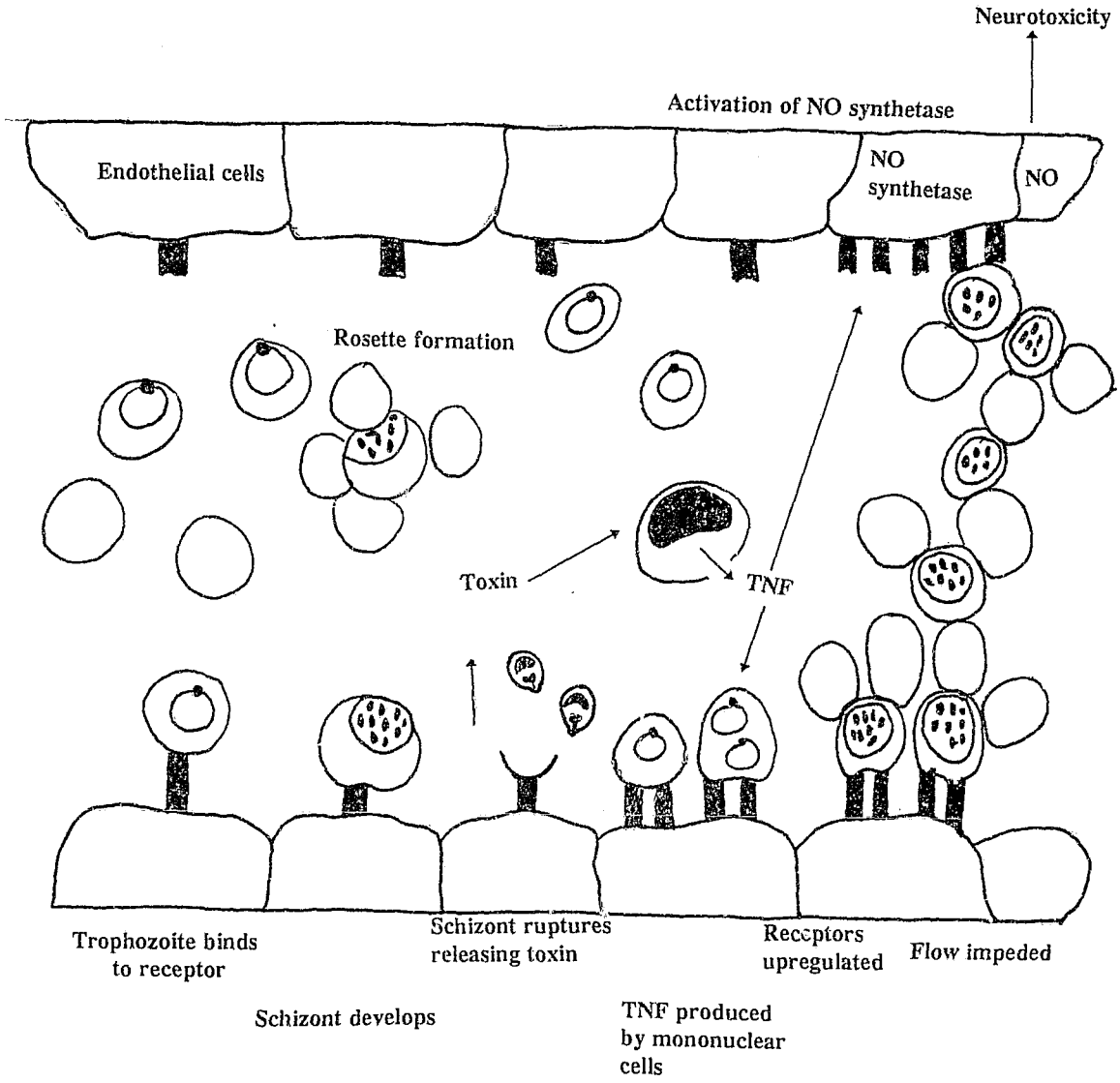


Figure 8: **Schematic representation of the pathogenesis of cerebral malaria**

(b) *Haemoglobin degradation in the parasitised erythrocyte*

Haemoglobin is degraded/digested in the *Plasmodium* digestive (food) vacuole,an acidic (pH~5) proteolytic compartment which is central to the metabolism of the parasite (Olliaro and Goldberg, 1995). Three proteases (2 aspartic and one cysteine) have been localised to the digestive vacuole . Together they account for the majority of haemoglobin degradation (Goldberg et al, 1991), which is illustrated in Figure 9. The aim of this haemoglobinolytic process is a source of amino acids which are required for parasite metabolism and growth. As a by-product of haemoglobin degradation an iron-rich heme group (ferriprotoporphyrin IX) is released. This is toxic to the parasite and is effectively removed by polymerising these residues into pigmented crystalline structures called haemazoin (Sullivan and Meshnick, 1996; Warhurst, 1995). The formation of haemazoin is believed to occur via haem polymerase activity, although some authors suggest that this is achieved independently of enzymatic activity (Dorn et al, 1995). During haemoglobin degradation and haemozoin production, there is some liberation of haem iron. This becomes oxidised from the ferrous^{2+} to the ferric^{3+} state with the generation of superoxides (O^{-}_2). These reactive oxygen species are neutralised by being converted firstly to hydrogen peroxide via a host derived superoxide dismutase, and then to water (H_2O) via the action of a catalase (CAT). Any free iron that is released is utilised by the parasite. Iron is an essential component for some *Plasmodium* enzymes e.g ribonucleotide reductase and cytochromes (Olliaro and Goldberg, 1995).

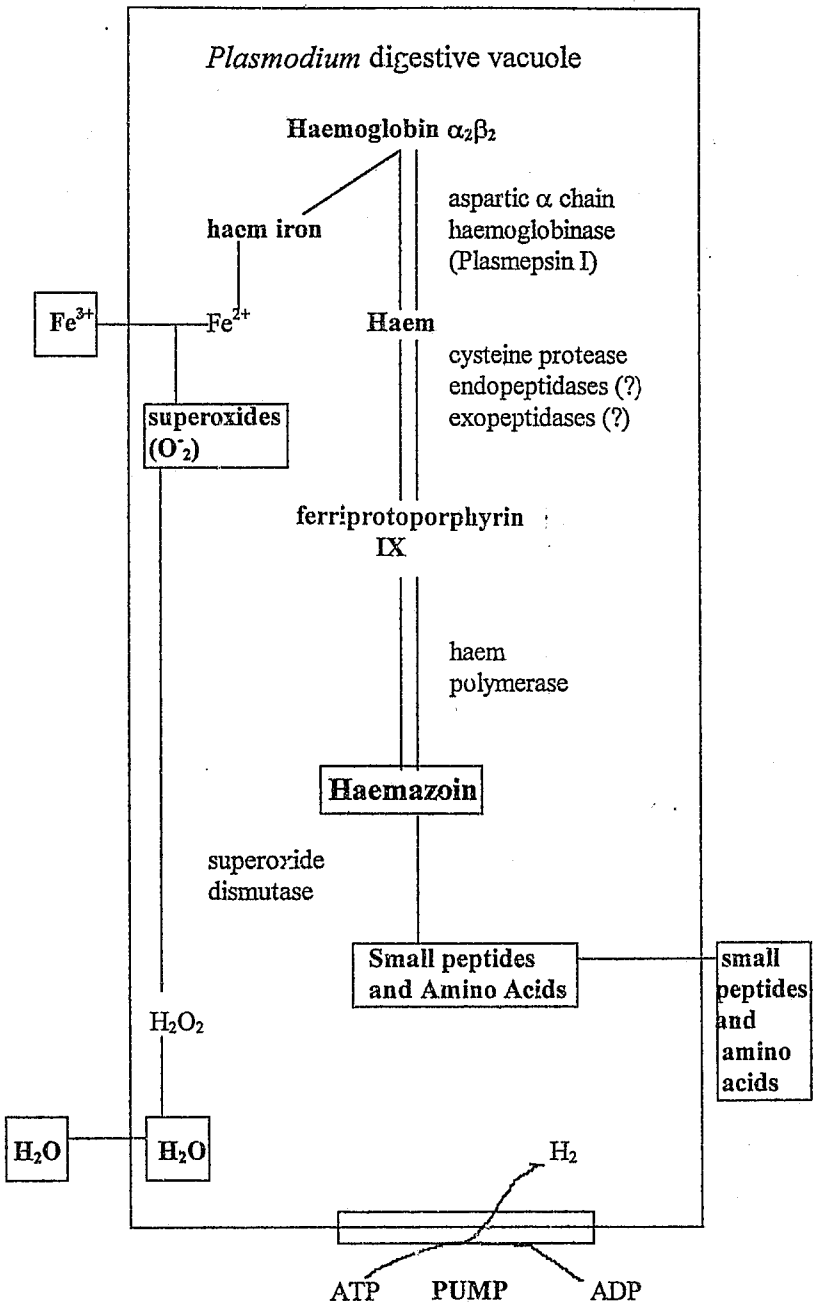


Figure 9: Proposed pathway of haemoglobin degradation

1.4 THE CLINICAL PRESENTATION AND PATHOPHYSIOLOGY OF *PLASMODIUM FALCIPARUM* MALARIA

Clinically malaria causes non-specific symptoms often manifesting initially with a flu-like illness comprising of fever, malaise and headache. Table 3 summarises these commonly presenting features. In many parts of the world severe and complicated malaria is the most common clinical presentation and cause of death. Table 4 lists the defining criteria for severe / complicated disease. The discussion that follows concentrates on a few symptoms and signs that have a major contributing effect on the morbidity/mortality associated with malaria.

1.4.1 Fever

The best or well known symptom of malaria is the febrile paroxysm (Warrell, 1993; Murphy and Oldfield, 1996). This starts with a rise in temperature usually exceeding 39°C. Despite this rise in body temperature the patients often develop a feeling of cold which is associated with shivering (cold stage). Peripheral vasoconstriction occurs with the development of cold, dry, pale and cyanosed extremities. The rigors can last from between 15 minutes up to 1 hour. The patient is soon engulfed in a feeling of warmth (hot stage) as peripheral vasodilation starts . The feeling is described as 'flushed' or 'burning'. During this phase which can last from 2-6 hours the patients may become confused /delirious and experience severe throbbing headaches as temperatures peak at up to 41°C. The fever declines over the next 2-4 hours as the patient breaks out in a drenching sweat with defervescence (sweating stage). The total duration of a typical attack is between 8-12 hours, at the end of which the patient is left exhausted (Warrell, 1993).

Table 3: **Symptoms and signs of uncomplicated malaria** (in order of decreasing frequencies)

Adapted from Warrell et al, 1990

Symptoms:	Signs:
Fever Chills Headache Fatigue/malaise Sweats Myalgia *Gastrointestinal Symptoms:* Nausea Vomiting Abdominal pain Diarrhoea *Respiratory Symptoms:* Pharyngitis Cough	Hyperpyrexia (>40°C) Splenomegaly Hepatomegaly Jaundice

Fever and the febrile paroxysms coincide with rupture of the erythrocytic schizont. Francis and Warrell (1993) suggest that these symptoms are the result of cytokines (potent endogenous pyrogens) released from macrophages into the circulation at the time of schizont rupture . These cytokines act on the hypothalamus' thermoregulatory centre initiating the physiological responses that cause fever viz. shivering, peripheral vasoconstriction and increased metabolic rate. This is mediated via the action of Prostaglandin E_2 (Kwiatowski, 1995).

1.4.1.1 Cytokines

1.4.1.1.1 Tumour Necrosis Factor α (TNF α)

TNF α is produced and secreted by activated mononuclear cells like macrophages and monocytes, in response to parasite derived proteins and substances (malaria toxins)

released by the rupturing schizonts (Jones and Hoffman, 1992; Kwiatowski, 1995; Mendis and Carter, 1995). A number of studies have recorded a strong positive correlation between plasma TNF levels and severity of disease e.g cerebral malaria (Jones and Hoffman, 1992; Kwiatkowski, 1995; Taylor-Robinson, 1995a) Several mechanisms have been postulated as to how TNF might promote cerebral disease:

(i) Upregulation of adhesion molecules e.g expression of ICAM-1 on the vascular endothelium. (see section 1.3.1.2 (iii)).

Table 4: Severe manifestations and complications of falciparum malaria.

Defining Criteria of severe disease
Cerebral Malaria*
Severe normocytic anaemia (Hb < 5 g/dl)[WHO]
Renal failure (urine output < 400ml/24hrs in adults; < 12mg/kg/24hrs in children; serum creatinine >265µmol/l (> 3.0mg/dl))[WHO]
Pulmonary oedema
Hypoglycaemia (glucose < 2.2 mmol/l ; < 40 mg/dl)[WHO]
Circulatory collapse/shock (systolic blood pressure < 50mm Hg in children ; < 70 mm Hg in adults; cold clammy skin)[WHO]
DIC[a]/ spontaneous bleeding
Generalized convulsions (repeated) - > 2 observed within 24 hrs
Acidaemia (arterial pH < 7.25)[WHO]
Acidosis (plasma bicarbonate concentration < 15 mmol/l)[WHO]
Haemoglobinuria
Other manifestations
Impaired consciousness (rousable)
Hyperparasitaemia (> 5% parasitaemia in non immune patients)[WHO]
Hyperpyrexia (rectal temperature > 39°C)[WHO]
Jaundice (detected clinically or by definition - bilirubin level > 50µmol/l; > 3.0mg/dl)[WHO]
Prostration /extreme weakness (no obvious neurological explanation)

* see Table 5

[a] Abbreviations: DIC, disseminated intravascular coagulation

Warrell, 1993

(ii) Enhancing the production of Nitric Oxide (NO) by inducing the enzyme nitric oxide synthase (NOS). This cytokine induced NOS has no negative feedback mechanism which implies that the generation of NO can reach concentrations that are 1000-fold greater than physiological levels. In addition the 'plugging' of the vessels, concentrates the source of the NO within the cerebral microvasculature, resulting in disturbed neurotransmission and ultimate neurotoxicity (Anstey et al, 1996).

The clinical manifestation of raised TNF levels namely the fever has been shown experimentally to have an inhibitory effect on parasite growth thereby regulating parasite density (Kwiatkowski, 1995). This has been postulated to be an early non-specific host mechanism that regulates parasite numbers before specific immune mechanisms intervene (Mendis and Carter, 1995). Other cytokines with pyrogenic properties detected in malaria patients include IL-1β, IL-1α, IL-6 and macrophage inflammatory protein -1 which are produced by monocyte / macrophage type cells; lymphotoxin α (LTα) produced mainly by lymphocytes. It is difficult to assess the exact contribution to the clinical manifestation of malaria by these cytokines as their activity/regulation is not as simple to measure as that of TNF (Kwiatkowski, 1995).

1.4.2 Cerebral Malaria

Cerebral malaria is one of the complications of severe malaria that is most feared, accounting for approximately 10% of all cases of falciparum malaria admitted to hospital (in many parts of the world) (Warrell, Molyneaux and Beales, 1990) and is often fatal. Neurological sequelae have been described in recovering patients, especially children, and vary in severity. As focal signs and neurological symptoms like impairment of consciousness with or without fever may result from other central nervous system

infections or even CNS vascular disease, a 'strict' definition of cerebral malaria has been recommended (Warrell et al, 1982). In clinical practice however, any patient (with a headache, neck stiffness, drowsiness, febrile convulsions or focal neurological signs) with any degree of impaired consciousness who may have been exposed to malaria , should be treated for 'cerebral malaria' with some urgency. Table 5 summarises the important clinical manifestations of cerebral malaria in adults and children.

`1.4.2.1 The pathophysiology and pathology of Cerebral Malaria

The exact mechanism remains a topic for debate; early hypotheses were based on pathological observations made in animal models or in fatal cases of falciparum malaria (Warrell et al, 1990).

~ *the 'sludging' hypothesis*: PRBC adhering to each other would reduce and eventually stop the flow of blood through the cerebral capillary bed. The subsequent pathology was the result of the obstructed microcirculation and/or the release of unidentified toxins from the malaria parasites.

~ *the 'permeability' hypothesis*: In severely ill animals (malaria infected rhesus monkeys) the primary abnormality was postulated to be an increase in the cerebral capillary permeability (which was mediated by various kinins) with leakage of plasma from the microcirculation which resulted in the development of cerebral oedema, and local haemoconcentration. This theory was the basis for the use of corticosteroids in cerebral malaria. Cerebral oedema resulting from 'leaky' capillaries does not appear to be a consistent,or even common ,feature of cerebral malaria. It is possible that this is a post mortem observation,resulting from agonal events occurring prior to death e.g hypotension, severe acidosis etc.

Table 5: The signs and symptoms of cerebral malaria

Sign or symptom	Adult	Children
Duration of symptoms before neurological manifestation	prodrome of several days of fever and non-specific symptoms	acute onset of symptoms often < 2 days
Neck stiffness	Common	Common
Abnormal posturing:		
opisthotonus	in severely ill patients	in severely ill patients
decorticate	Common	Common
decerebrate	Common	Common
Convulsions	Common in 1/2 of adults; this may indicate cerebral involvement or hypoglycaemia (usually generalised seizures)	Very common ; usually indicates cerebral involvement or hypoglycaemia but it may be a non-specific symptom of fever (usually generalized but persistent focal seizures have been observed)
Coma	various levels/degree of unconsciousness have been observed (these are assessed using the Glasgow Coma Scale)	unrousable persisting coma may follow a generalized convulsion
Papilloedema	Uncommon	Uncommon
Retinal haemorrhages	Common	Common
Disorders of conjugate gaze	Common	Have been observed in African children
Corneal /eyelash reflexes	Usually intact	May be absent
Hypoglycaemia	Uncommon (usually quinine induced)	Common (usually pre-treatment) -especially in children < 3 years. Is often associated with convulsions and deep coma. May be a feature of hyperparasitaemia
Neurological sequelae after cerebral malaria	Uncommon (but the following have been described: cranial nerve lesions/neuropathies;extra-pyramidal signs/Guillain Barre like syndrome; reactive psychosis in the recovering patient.	Occur in about 10% of cases. Hemiplegia is common; others include: cortical blindness; ataxia;behavioural disturbances; reactive psychosis in recovering patients.

Adapted from Warrell et al, 1990

~ *the 'mechanical' hypothesis*: (a) Red blood cells need to undergo some degree of deformability inorder to pass through the capillary vasculature. Infected erythrocytes have been shown to become less deformable - this appears to be directly proportional to the maturity of the intracellular parasite. One of the consequences is microvascular obstruction, (b) Cytoadherence and rosetting are also important mechanisms in the obstruction of cerebral vasculature. These concepts have been discussed and illustrated in detail under section 1.3.1.2 (a) (iii) and will not be elaborated further here.

(c) The role that cytokines (esp TNFα) play in the pathogenesis of cerebral malaria has already been discussed at the beginning of this section (1.4.1.1).

~ *the 'immunological' hypothesis*: Immune mechanisms have been described in the pathogenesis of certain severe disease manifestations of malaria e.g glomerulonephritis and cerebral lesions, in rats and mice (Warrell et al,1990). In 1978, Toro and Roman proposed that the neuropathological findings evident in cerebral malaria were due to an immune complex mediated vasculitis of the cerebral vessels resulting in perivenous demyelination. Histological evidence of vasculitis ,in fatal cases of falciparum malaria however, is absent (Warrell et al, 1990).

1.4.3 Anaemia and thrombocytopenia

Several haematological changes have been described in malaria, these include anaemia, neutropenia, reactive lymphocytosis, monocytosis, eosinopenia, and thrombocytopenia (Abdalla, 1990; Warrell et al, 1990). Of these, anaemia is often associated with considerable morbidity especially among children and pregnant women. Malaria causes varying degrees of anaemia ranging from life-threatening levels to levels slightly below normal values. Severe anaemia (Hb < 5g/dl or haematocrit < 15%) is a life-threatening

complication of malarial infection, and its development is often related to the degree of parasitaemia and schizontaemia (Warrell et al., 1990). The pathogenesis of malarial anaemia will be elaborated further in the discussion. In patients with acute malaria, severe anaemia often contributes to cardiovascular signs (gallop rhythm, hepatomegaly and pulmonary oedema) and cerebral signs (confusion, restlessness and coma).

Thrombocytopenia is a common finding in falciparum malaria and not necessarily a feature of severe malaria. In most cases it is not associated with bleeding. Significant bleeding usually occurs in association with other haemostatic abnormalities for example when thrombocytopenia is present in the setting of a disseminated intravascular coagulopathy (DIC). Possible causes of a decreased platelet count include reduced platelet survival due to peripheral consumption, as is found in a DIC, or due to immune mediated mechanisms, increased splenic uptake/sequestration and decreased or ineffective thrombocyte production, most likely from infection related marrow suppression (Warrell et al, 1990).

1.4.4 Renal dysfunction

This complication appears to be commoner in adults than in children. There are several distinct patterns of renal involvement in malaria: a transient and self-limiting glomerulonephritis which appears to be associated with the presence of immune complexes within the glomeruli and circulating in the serum and which is rarely of clinical significance, and that of an acute tubular necrosis, the mechanism of which is not clearly understood (Francis and Warrell, 1993). Cytoadherence of PRBC in the glomerular capillaries may play a contributing role. In addition many of the patients are dehydrated at presentation, and often renal function is restored by simply rehydrating.

1.4.5 Hypoglycaemia

Hypoglycaemia is an important manifestation of falciparum malaria, with more than 5% of children with severe malaria presenting with suboptimal glucose levels, on admission to hospital. It is also commonly seen in pregnant women with malaria and in patients with severe disease and hyperparasitaemia. There are several possible causes (Warrell et al, 1990):

(a) Drug induced hypoglycaemia

(b) Increased glucose consumption by the host and the parasite

(c) Impaired gluconeogenesis or glycogen depletion

1.5 IMMUNITY IN MALARIA

Two systems of immunity arise when a host is exposed to an infectious agent: a) innate/natural immunity and b) acquired/specific immunity (Fearon and Locksley, 1996).

Innate/natural immunity

Innate/natural immunity seems to be selected through evolutionary pressure. Individuals exhibit variable susceptibility to infection, and some of this resistance is inherited. It is safe to say that most of the red cell defects recognised today are polymorphisms which have arisen from exposure to malaria e.g HbS, α and β thalassaemia etc.(see also section 1.3.1.1 (c) (iii)). The protective effect of alpha and beta thalassaemia against malaria seems established... or is it ? Recently an article appeared in *Nature* that showed a higher incidence of malaria in a population of homozygous alpha thalassaemic Melanesian children compared with normal or heterozygous children (Williams, Maitland and Bennett et al, 1996). Is the malaria hypothesis wrong ? (Yuthavong and

Wilairat, 1997). The authors felt that the alpha^{+} gene (in homozygous children) gives rise to increased susceptibility to malaria in early childhood (under 5 years of age), which may result in improved immunity in later years. In this group of children a high incidence of both *P.falciparum* and *P.vivax* was noted. The questions that are now being raised include whether infection by *P.falciparum* in alpha thalassaemic children in the 1st four years of life give protection against more severe attacks later in life ? (Yuthavong and Wilairat, 1997).

Maitland et al (1997) have introduced another hypothesis. They postulate that increased susceptibility to *P.vivax* infection in homozygous alpha^{+} thalassaemic children may induce crossprotection against subsequent severe *P.falciparum* malaria. This theory may be supported by the observation that homozygous children older than five years of age had a reduced incidence of *P.falciparum* infection compared to the normal and heterozygote group. This theory needs to be tested by looking at large scale epidemiological studies in appropriate populations (i.e those naturally exposed to both malarial species), since volunteer studies are not ethically feasible.

To complete the list of disorders/polymorphisms that have been implicated in resistance to malaria and especially to *Plasmodium falciparum* we have to include recent evidence that suggests the effect of HLA variation on malaria susceptibility (Hill, 1992). Associations were found between two HLA classes: HLA Bw53 and HLA DRB1*1302. These were shown to strongly protect individuals from cerebral malaria and severe malarial anaemia.

Acquired/Specific Immunity

Immunity to malaria parasites involves both cellular and antibody mediated mechanisms. The cellular and molecular bases of acquired immunity in malaria is not well understood,

but most people would accept that the development of acquired immunity involves an initial cell mediated phase that is mediated through macrophage/neutrophil activation and by the release of cytokines such as interferon gamma (IFN γ) and tumour necrosis factor alpha (TNF α); the effects of TNF α in the pathogenesis of malaria have been discussed under section 1.4.1.1. This cellular response is controlled by $CD4^+$ T helper (Th1) cells, and is often associated with the production of oxygen and nitrogen intermediates. The effect of this mechanism is to limit parasite replication.

This is gradually replaced by a $CD4^+$ T helper (Th2) cell mediated response. This is predominantly an antibody-dependent mechanism. Antibody has been shown to prevent red blood cell invasion, to increase the removal of merozoites and infected erythrocytes by monocytes and even to have a role in the displacement of PRBC from the endothelial cells of the brain capillaries. After the initial IgM production following acute infection, there is an isotype switch towards immunoglobulins of the IgG1 and IgG3 subclasses (Ferreira, Kimura, De Souza and Katzin, 1996). These are cytophilic antibodies and have been shown to act via an association with the monocyte/macrophage systems (Figure 10) to effect parasite-killing mechanisms (Druilhe and Pérignon, 1997) . Antibody-mediated protective immunity is gradually acquired over a long period of time (Baird, 1995), and generally requires uninterrupted exposure to the infectious antigen. One of the reasons why antibody-mediated immunity takes time to develop and become effective is because the parasite has been shown to display considerable antigenic variation/diversity (Pologe and Ravetch, 1986; Nowak, 1995), and the host has to experience a large number of strains and variants before an effective immunity develops.Protective immunity, however, may fail to develop and may be rapidly lost after it has developed, with interrupted exposure, like with seasonal malaria,or if there is

extended travel out of an endemic area. The half life of the IgG subclasses is variable: IgG3 has a $t\frac{1}{2}$ of 9-10 days, the remaining subclasses have a half life of 23-25 days (Ferreira et al, 1996). The mechanisms that effect immunity following malaria infection have been studied mostly in murine models, however the model that is illustrated in Figure 11, can easily be applied to the human counterpart.

The detailed mechanisms that prevent parasites multiplying exponentially in humans are still not well understood, however it has been suggested that the severity of the infection especially with *Plasmodium falciparum* may be determined by the degree of activation of the $CD4^{+}$(Th1) T cell mechanism (Taylor-Robinson, 1995b). The mechanisms described above, may result in a state of protection that prevents the occurrence of clinical symptoms and high parasitaemias.

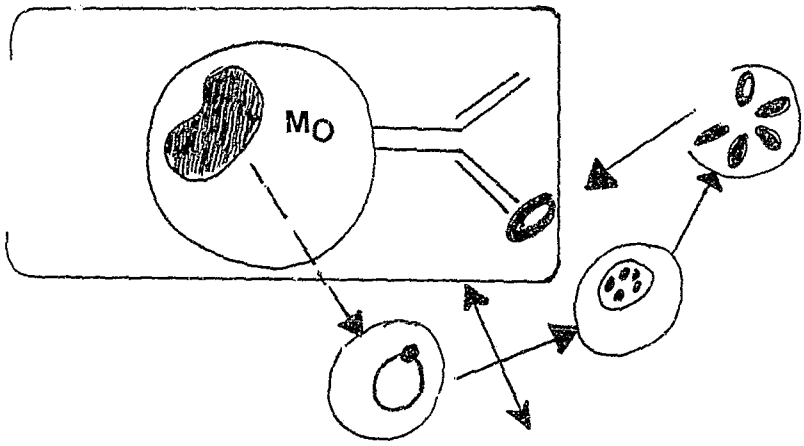


Figure 10: **Antibody-dependent monocyte-mediated mechanism of growth inhibition of *P.falciparum*. [Cytophilic antibodies crosslink monocytes, MO to merozoites and this leads to release of monocyte derived mediators (dotted arrow) which block intraerythrocytic parasites (bold arrow)].**

Reproduced from Druilhe and Pérignon, 1997

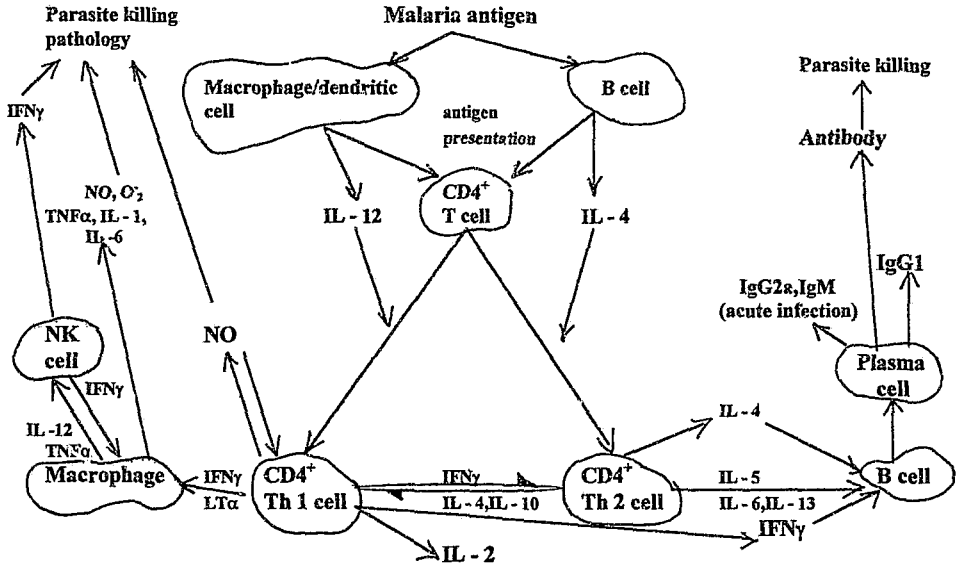


Figure 11: Schematic diagram of the cell mediated and antibody-dependent mechanisms of protective immunity to asexual erythrocytic stages of *Plasmodium* infection.
Reproduced from Taylor-Robinson, 1995b

No matter what the duration of exposure is, a few parasites will still be found circulating in the blood. In areas of high malarial transmission more than half the adults have been shown to have a low grade parasitaemia (Druilhe and Pérignon, 1997). The cause of this low grade 'chronic'infection is thought to be the presence of antigenic variation. The parasite appears to successfully evade the hosts immune system by expressing a new variant type. While the hosts immune mechanisms are being evaded parasite densities can fluctuate between very high and undetectable, until the new immune responses are raised.

The above discussion regarding malaria immunity has been simplified. It is becoming increasingly clear that there is more than one distinct defence mechanism against blood stages, and there appear to be many polymorphic and variant antigens that can evoke

specific immune responses; these antigens are also used by the parasite to escape the direct effects of the antibody-mediated mechanisms. The final outcome of continous antigenic exposure is a compromise between the parasite and the host with the establishment of a 'chronic' state.

The crusade for the development of the perfect vaccine, may bring to light more definitive defense mechanisms that are elicited in the war between host and parasite.

1.6 MALARIA IN PREGNANCY

Pregnant women (especially primigravidae) have an increased susceptibility to parasitic infections like malaria (Warrell et al, 1990; Francis and Warrell, 1993), among a number of other pathologies. This increased risk is associated with miscarriages, stillbirths, and placental infection that is often the cause of low birth weight babies. The effects of malaria on pregnancy are summarised in Table 6.

1.6.1 Pregnancy and immune suppression

Pregnancy is a time when important physiological events occur in the mother, which help the foetus to adapt to a new environment, and prevent its rejection. This includes a degree of immune suppression which is characterised primarily by a depression of cell mediated immunity/type 1 cytokine responses but impairment of humoral immunity/type 2 cytokine responses has also been implicated (Fievet et al, 1995; Menendez, 1995; Smith, 1996).

Humoral immunity

IgG titres appear to fall as pregnancy advances. This however seems to be a relative decrease and is probably related to (a) the haemodilution that occurs in the 2nd trimester

and (b) transplacental transfer of IgG from the maternal to the foetal circulation. The data regarding malaria-specific antibodies is contradictory; a reduction in malaria - specific antibody titres has been reported in parasitaemic women (Deloron et al, 1989; Mvondo et al, 1992). Other investigators, however have failed to detect any difference in malaria antibody titres between pregnant and non-pregnant women (Riley et al, 1989; Rasheed et al, 1992), nor has there been a difference in malaria specific antibody titres demonstrated among women of different parity groups.

Cell-mediated immunity

Pregnancy is associated with the increased production of many proteins including hormones that are responsible for maintaining the foetus (Guyton, 1981). Among these cortisol and oestrogens have been associated with suppression of cell-mediated events *in vitro*. Interestingly these two hormones are found in higher levels in primigravidae compared with multiparous women (Menendez, 1995). The placenta appears to be a site for preferential parasite sequestration and development. An explanation for this has been based on the local role of immunosuppresive factors produced by the placenta, like the oestrogens. This suppression in local immunity appears to decrease as the pregnancy progresses, possibly due to decreased production of hormone from the infected placenta.

1.6.2 Epidemiology of malaria in pregnancy

A few important features regarding the epidemiology of malaria have been summarised below (Menendez, 1995):

1. Pregnant women appear to acquire malaria more frequently and are at an increased risk of developing severe malaria, compared to non-pregnant women.
2. The level of pre-pregnancy immunity usually determines the frequency and severity of the malaria infection. Women from areas where malaria transmission is low are likely to have poorly aquired immunity prior to pregnancy. Women from highly endemic malaria areas have significant protective immunity and the effects of the infection are usually less severe.
3. Among non-immune women the susceptibilty to infection is increased regardless of parity. In holoendemic areas where the level of protective immunity is expected to be high, primigravidae appear to be more at risk (for reasons already described in section 1.6.1).
4. Although the effects of genetic factors e.g sickle cell trait, on malaria have been well described, the importance of these in the setting of pregnancy are contradictory.
5. Nutritional factors like the maternal iron and folate status may affect susceptibility to malaria infection.

Menendez (1995) puts it in a nutshell - "...effects of malaria in the pregnant women and the foetus,...have been well described, but there are still many aspects of the relationship... that are poorly understood."

Table 6: The effects of malaria on pregnancy

Effects of Malaria	Malaria Endemicity	
	Low	High
On the mother		
Severe disease,including death	not infrequent	indirectly through anaemia
Acute disease/anaemia	frequent (all parities)	frequent (primigravidae)
Hypoglycaemia	not infrequent	not known
Placental infection	infrequent	frequent (primigravidae)
On the foetus		
Abortion/perinatal death	not infrequent	not known
Intra Uterine Growth Revardation	frequent (all parities)	frequent (primigravidae)
Prematurity	not infrequent	not known
Congenital infection	not infrequent	infrequent
On the infant		
Reduced birth weight	not infrequent	frequent
Increased risk of death	not infrequent	not infrequent
Increased susceptibility to malaria	not infrequent	frequent

Adapted from Menendez, 1995

1.7 PREVENTION AGAINST MALARIA

Researchers and malariologists have spent more than 20 years trying to develop methods for malaria control (WHO, 1993). However, in some parts of the world e.g Tropical Africa, the malaria situation is worsening. As the number of cases detected every year continue to increase, measures for the prevention, diagnosis , and treatment gain greater importance.

1.7.1 Vector Control

Controlling the insect vector has been an effective way of reducing the transmission of disease - causing organisms to human hosts. A variety of methods have been employed (Curtis, 1994; Luxemburger et al, 1994; Crook and Baptista, 1995), including the elimination of breeding sites with chemical insecticides (Onori et al, 1993). Insecticide use however, is not without hazard and the dangers of continuous exposure to these chemicals by the individuals spraying, and those exposed to the fumes (residents of sprayed homes) are receiving considerable attention (Kreiss et al, 1981; Bouwman et al, 1994; Bouma and Nesbit, 1995). In addition the emergence of resistance to these chemicals has necessitated the development of new compounds with additional costs, and possible detrimental effects on the environment. Parasitologists have therefore needed to assess alternative methods to suppress vector populations, or to change their ability to transmit disease- causing organisms.

Recently researchers have identified the insect vector's genome and other sites e.g. the insect midgut as appropriate molecular targets for genetic manipulation. Insect vector populations may be created that have been "programmed" with certain characteristics e.g insecticide susceptibility, temperature sensitivity or altered transmission of disease causing parasites (Billingsley, 1994; Crampton 1994).

1.7.2 Antimalarial Vaccines

Malaria control programmes and treatment strategies are proving to be less effective than anticipated, as evidenced by the increasing malaria transmission worldwide. As our options to control this disease appear to be running out, the need for the development of a malaria vaccine has become a priority. Our evergrowing knowledge of the immune

responses elicited in the host by the parasite allows us to believe that vaccines to malaria are biologically possible (refer to section 1.5). In fact, it has already been demonstrated that vaccination can protect humans against malaria. In the early 1970's, volunteers who were previously unexposed to malaria were exposed to the repeated bites of irradiated mosquitoes that had been infected with *P.falciparum* or *P.vivax*. This produced a solid immune response (Clyde et al, 1973; Clyde et al, 1975). Clearly the method of immunisation is not a practical one to use on a large scale. In order to achieve a worldwide vaccination program the candidate vaccine would have to be either chemically synthesised or be produced using recombinant DNA technology (Jones and Hoffman, 1994).

In the late 1980's Patarroyo and colleagues introduced the "First Malaria Vaccine" (Patarroyo et al, 1988; Valero et al, 1993; Tanner et al, 1995). The vaccine is a chemically synthesized polymeric hybrid protein of 150 kDa - SPf66, which was shown to induce partial or complete protection in experimentally infected monkeys (Patarroyo et al, 1987). Preclinical and clinical human trials established that the innoculum was safe and immunogenic, and appeared to have some protective efficacy against malaria in areas of low endemicity (Valero et al, 1993). In Africa, in an area of high malaria transmission, the overall efficacy of the vaccine was 31% (Alonso et al, 1994). The vaccine did not demonstrate any pre-erythrocytic immunity,but rather acted as an asexual blood stage vaccine, reducing the incidence of clinical malaria. Recent clinical trials of this vaccine however have shown disappointing results, and the overall conclusion is that SPf66 may not effectively protect against clinical malaria (Nosten et al, 1996).

1.7.2.1 Vaccine strategies

1.7.2.1.1 Target populations

The criteria for the success of a malaria vaccine are often different for different groups of individuals exposed to the infection. It is therefore important to identify the target populations for whom the vaccine is being designed. Infants and children in malarious (endemic) areas will require a vaccine that will reduce the morbidity and mortality associated with the malaria but at the same time will allow the development of natural immunity through repeated re-infection. Vaccines for non-immune individuals who are exposed to malaria transiently, e.g tourists, business travellers will be required to prevent asexual infection completely.

1.7.2.1.2 Vaccine design

The life cycle of the malaria parasite (refer also to section 1.3.1) has certain stages which appear to be more prone to immune attack than others. Vaccines incorporating antigens from these stages would theoretically be capable of blocking infectivity.

Table 7 summarizes the candidate antigens from the various stages in the malaria parasite's life cycle, highlighting some important properties.

1.7.2.1.2a *Cytoadherence*

An important factor responsible for the severe pathology of *P.falciparum* is the ability of the PRBC to adhere to endothelial cells. A vaccine that induces antibodies to the components of adhesion (e.g. knoblike projections) could eliminate attachment to the endothelium and reduce the development of cerebral malaria and its associated morbidity and mortality. Several proteins have been implicated in the mechanisms of cytoadherence and these have been reviewed under section 1.3.1.2.

Table 7: **Candidate antigens from the malaria life cycle: - targets for the development of an antimalarial vaccine.**

Stage	Antigen, Location,Properties	References
Sporozoite	**Circumsporozoite(CS);** expressed on the surface of the sporozoite; has a highly immunogenic area of repeated amino acid sequences $(NANP)^a$; [a number of vaccine trials have reported mixed results]. **Sporozoite surface protein 2 (SSP-2);** this is a 140 kDa protein; has a multiple tandem repeat sequence of six amino acids ($NPNEPS)^a$; [mice immunised with this protein were shown to develop a partial immunity which was CD8+ dependent].	Khusmith et al, 1991 Reber-Liske et al, 1995 Sherwood et al, 1996 Stoute et al, 1997
Liver stage	**Liver stage specific antigen 1 (LCA-1);** expressed on the surface of hepatocytes; a 230 kDa protein that contains an immunogenic 17 amino acid repeat.	Guerin-Marchand et al, 1987 Aidoo et al, 1995
Pre-Erythrocytic	**Merozoite surface protein 1(MSP-1);** a 195 kDa protein synthesized at the schizont stage; precursor of four major polypeptides found in a non-covalently bound complex on the merozoite surface; polymorphic antigen, also has conserved regions; a small 19 kDa fragment ($MSP\text{-}1_{19}$) is derived after RBC invasion; [this fragment is protective against clinical malaria]. MSP-1 has been used in fusion/hybrid proteins and has demonstrated protection from experimental malaria infection in animals. **Merozoite surface protein 2 (MSP-2);** has not been shown to successfully protect against infection in animal models.	Enders et al, 1992 Hui et al, 1992 Pasvol et al, 1993 Jones and Hoffman et al 1994 Egan et al, 1996
Erythrocytic	**Erythrocyte binding antigen 175 (EBA-175);** a 175 kDa protein expressed on merozoites and has been localised to the micronemes at the apical end. **Ring infected erythrocyte surface antigen (RESA);** a 155 kDa protein localised to the dense granules of the merozoite; after invasion the protein is expressed on the RBC surface; has regions of rpeated amino acids - an octapeptide $(EENVEHDA)^a$ and tetrapeptide $(EENV)^a$ **Serine repeat antigen (SERA);** a 113 kDa protein characterised by a stretch of 37 serine residues, found in the parasitophorous vacuole and is released when PRBC lyse; [shown to induce partial protection in Saimir monkeys].	Howard and Pasloske, 1993 Perrin et al, 1984

[a] Abbreviations (Table 7): A -Alanine; D- Aspartate; E - Glutamine; H - Histidine; N - Asparagine; P -Proline; S - Serine; V - Valine.

1.7.2.1.3 Transmission-blocking vaccines

Most treatment protocols are unable to treat or eliminate the sexual stages of the parasite, that are largely responsible for the continued transmission of malaria from the host to the vector. Here is another target that would fit within the scope of vaccine development. If antibodies could be generated in the host against antigens of the sexual stages, these could be transmitted to the mosquito during a blood meal, and in this way disrupt sexual reproduction. The most obvious targets include: antigens on the surface of gametocyte-infected red blood cells; molecules on the plasma membrane of the extracellular gametocyte/gamete/zygote and molecules associated with the ookinete that interact with the insect midgut (Sinden, 1997). These vaccines would not be capable of protecting the vaccinated individual from infection, however if sufficient individuals within an endemic community were to be immunised, the cycle of transmission could be broken thus confering protection against subsequent infections.

1.7.2.1.4 Vaccines of the future

To date antigenic material for the development of vaccines has been derived primarily from: (1) live attenuated, or killed forms of the whole organism; and (2) defined proteins or recombinant protein components of the organism, most likely derived from biochemical purification or by genetic engineering (Cheung et al, 1986; Patarroyo et al, 1987; Patarroyo et al, 1988; Chatterjee et al, 1995). The major problem with the live vaccines, however, is the risk that the organism may revert to a pathogenic form. To overcome this problem without compromising the T cell mediated responses that the

conventional vaccine methods try to induce, researchers found a novel way of introducing the desired immunogen into the host. A DNA (or RNA) construct, encoding the protein of interest is delivered to the cells of the organism to be vaccinated. It is taken up and expressed by the host cells,whereupon it induces a protective immune response. Several questions however need to be answered before this type of DNA vaccine is available for human trials, like what is the best method of administration of the relevant construct, what are the advantages over conventional vaccines, and are there any safety issues to be considered ? (Waine and McManus, 1995). There was some concern about the generation of anti-DNA antibodies,in the mice DNA vaccine trials, and the possible development of autoimmune disease.

Gilbert and Hill (1997) recently reviewed the use of viral coat proteins to produce hybrid particles carrying *Plasmodium* sequences. These protein particles were found to be highly immunogenic in animal studies , and may represent a new type of vaccine that may be effective against malaria. Several types of particles have been developed as carriers of *P.falciparum* sequences, including Hepatitis B surface antigen (HBsAg) and Hepatitis B core antigen (HBcAg). These hybrid proteins are produced in microorganisms. In a recent phase I human trial, the protein particle vaccine RTS,S (HBsAg incorporating $(NANP)_{19}$ repeats of the CS protein) was tested in 17 volunteers, using a new adjuvant combination. All 17 subjects developed antibodies against the CS antigen. Seven individuals agreed to undergo repeated challenge, and 6/7 showed complete protection from the infection (Stoute et al, 1997). These results are impressive enough to persue along this avenue of new vaccine candidates.

1.7.2.1.5 Vaccine delivery systems

The component of the delivery system , the adjuvant, is a non-specific immunogenic mediator that often induces high antibody titres. The only adjuvant used at present is aluminum hydroxide (alum) but newer methods have been described that induce significantly higher antibody responses; these include the use of bacterial cell walls e.g mycobacterium; liposomes, oil emulsions and microencapsulation systems using biodegradable and free-flowing spherical particles are also showing promise (Eldridge et al, 1993). Other novel approaches include the use of recombinant live vectors and multiple - antigen/epitope peptide systems (MAPs/MEPs) (Chatterjee et al, 1995; Jones and Hoffman, 1994).

The degree of protection achieved with the various vaccine preparations has varied from partial to complete. Variations in the antigen preparations, immunization protocols, animal species, the parasite inoculum used, and the virulence of the parasite strains could account for the differences observed among the vaccination studies.

1.8 DIAGNOSTIC TECHNIQUES

1.8.1 Microscopy

The laboratory diagnosis of malaria depends on the demonstration of the parasite in thick films of peripheral blood (a screening tool) and the differentiation of the species and the determination of the parasitaemias is made on the thin films (Hira and Behbehani, 1984). Blood may be obtained by fingerprick or venipuncture [specimens are sent in an anticoagulant ethylenediamine tetra-acetate (EDTA)], and the slides made according to standard operating procedures (see Materials and Methods), are usually stained with a Giemsa stain . Most laboratories are able to offer this service on a 24 hour

basis and report the result of a thick film examination as soon as possible (ideally within one to two hours).The urgency placed upon this test often results in poor quality specimens with questionable interpretations. Laboratories must have an effective policy to assure malaria smears are made correctly and quickly (Hira et al, 1984). Despite careful handling +/- 10% of the specimens made over a year are classified as being of poor quality. When performed by a skilled microscopist,examination of stained blood films is a sensitive but laborious method. As few as 10 - 20 parasites /µl of blood can be detected.. Occasionally errors occur and among the common mistakes are confusing platelets or light spots and even debris in distorted red cells with parasites. Identifying the species of the parasite usually poses a challenge from the onset. If the clinical history is suspicious of malaria but the first slide is negative, the smears should be repeated every 6-8 hours for 48 hours. Blood should, if possible, be taken during or after pyrexia and before the administration of any antimalarial drugs. The parasitaemia is important to quantify as it has value as a predictor of clinical severity and prognosis (White et al, 1992). On thin smears parasites per 500 -1000 red blood cells are determined and converted to percent parasitized erythrocytes. The appearance of late trophozoites and schizonts in *P.falciparum* appears to correlate as a predictor of mortality (Silamut, 1993; Warhurst and Williams, 1996).

After therapy begins, the parasitaemia should ideally be monitored one to two times a day to assess the therapeutic efficacy of the drug regimen. This however is not always practical and a daily parasite count may need to suffice. Initially parasite levels may increase in the first 12-24 hours as the available antimalarial drugs do not usually inhibit schizont rupture and release of merozoites. The time from initiation of therapy to

repeatedly negative blood smears is known as the parasite clearance time (PCT). Most of the currently used drugs produce a PCT of 2 to 4 days (Murphy and Oldfield, 1996).

Although microscopic detection of the malaria parasite remains the gold standard of diagnosis, many techniques have been tried and tested in the attempt to find a quick, accurate, sensitive, early and cost effective method of diagnosis (McLaughlin et al, 1992). Below I have reviewed some old and new methods of parasitic detection.

1.8.2 DNA/RNA probes and hybridization for the detection of *P.falciparum* malaria

Plasmid borne repetitive DNA and RNA probes that have been labelled with radioactive isotopes have been described (Franzen et al, 1984; McLaughlin et al, 1985; Holmberg et al, 1987; McLaughlin et al, 1987; McLaughlin et al, 1991).

These tests appear to be highly sensitive, detecting parasitaemias of 0.0001% (+/- 40 parasites). Total genomic DNA appears to be 5 fold more sensitive than synthetic DNA probes. They have also been shown to be highly specific as each probe has sequences that recognise one malaria species and no crosshybridization was observed. This method is useful for detecting current infections, several hundred blood samples can be processed simultaneously and the procedure is completed within 24 hours. Despite this microscopy still remains more sensitive than the DNA probe in detecting low level parasitaemias (Lanar et al, 1989). The test can be modified to use fingerprick whole blood (Pollack et al, 1985; Barker et al, 1986), and has been adapted further to a nonisotopic enzyme linked synthetic DNA detection, using a rapid lysis and filtration

processing procedure (McLaughlin et al, 1988; Barker et al, 1989; McLaughlin et al, 1991).

In addition to DNA probes, rRNA sequences have been used as genetic markers for species or strain identification. Ribotyping has been used for pathogen identification among bacterial species to identify virulent strains (McLaughlin et al, 1992; Weiss, 1995). DNA from the pathogenic organism is digested with restriction endonucleases and then hybridised with rRNA or r DNA. The resultant band size polymorphisms provide a DNA fingerprint of the organism.

1.8.3 Serology

Serology is not used routinely as a diagnostic tool, as acute malaria is satisfactorily and more quickly detected on the blood films, however there are clinical situations in which this technique may be of benefit e.g in treated patients who no longer have demonstrable parasites in the peripheral blood, in infections with extremely low-level parasitaemias. The traditional role for serological testing remains in the epidemiological studies. Other applications include:

- Transfusion blood screening (Bruce-Chwatt, 1982; Espinal and Morales,1984).
- Retrospective diagnosis

1.8.3.1 ELISA (enzyme linked immunosorbent assay)

The surface of the *Plasmodium* sporozoite provides a target for immune responses from the host. There has been tremendous progress in the characterisation of the surface antigens of the sporozoite, in particular the circumsporozoite (CS) protein, which has been implicated as the target for host antibody responses (Jones and Hoffman, 1994;

Chatterjee et al, 1995; Gilbert and Hill, 1997; Nussenzweig and Zavala, 1997). Enzyme linked immunosorbent assays have been developed to detect antibody against a whole host of antigens, e.g the synthetic peptide $(NANP)_3$, derived from the repeating amino acid sequence on the surface of the CS protein (Campbell G et al, 1987), and a recombinant protein of glutamine rich residues of *P.falciparum* called GLURP (Dziegiel et al,1991). A major drawback of this assay is the variability of nonspecific reactions resulting in false positive results. Microtitre well formats for malaria show promise for uniform large scale monitoring of falciparum malaria in epidemiological studies, but further improvements in the technologies are necessary to improve costs and duration of the assay. Commercial kits are becoming available (CeLLabs Malaria IgG CELISA®-NSW Australia).

1.8.3.2 IFAT (indirect fluorescent antibody test)

The IFAT was developed more than 20 years ago but still remains one of the most widespread reference methods for the estimation of antibodies against *Plasmodium* specific antigens (Collins et al, 1964a; Collins et al, 1964b; Spencer et al, 1979). For this test antigen spots or thick films are made on glass slides. The antigen is usually derived from in vitro cultures of *Plasmodium* strains. The slides are then dried and stored frozen. Under these conditions the antigen can remain stable for years (Warhurst and Williams, 1996). Serum samples are then screened under various dilutions (1:20;1:40;1:80). A fluorescein-labelled anti-human immunoglobulin is used as a conjugate and the samples are analysed under a microscope that is equipped for FITC epifluorescence (Bush and Frean, 1996).This technique is however very time-consuming and subjective.

1.8.4 PCR (polymerase chain reaction)

The addition of PCR to the "molecular biology bag of tricks" has made a considerable impact on medical research as a whole. PCR offers maximum sensitivity for the direct detection of infectious organisms (Viriyakosol et al, 1995). In a recent review of molecular diagnosis of parasitic infections, Judith Weiss (1995) describes several techniques used in PCR assays for the optimisation of parasite detection. PCR proved to be significantly more sensitive than direct microscopic evaluation at detecting *P.falciparum* DNA in human blood,especially in samples with low-level parasitaemias, detecting many specimens that were negative by microscopy.

Another major advantage is the ability of PCR to detect mixed infections by analysis of polymorphisms encoding cell surface molecules that contain regions of repeat sequences. By modifying the technique PCR may provide invaluable information regarding phenotypic characteristics of various *Plasmodium* strains e.g. amplification of DNA from chloroquine resistant (CQR) and chloroquine sensitive (CQS) falciparum malaria infected blood, yielded different banding patterns (Kain and Lanar, 1991).

1.8.5 Antigen detecting tests

In the quest to develop the ultimate quick,accurate, sensitive, cost-effective and easy method of detection that will facilitate early diagnosis of malaria, a number of tests have been introduced in the last five years, all claiming to be the solution to any diagnostic problems (Figures 12 and 13). All these tests share a few basic features: (a) the identification of a *Plasmodium* species specific antigen, (b) they comprise of a non-automated ,easy to follow step-by-step procedure that requires very few skills to master the technique and is thus suitable for use in field clinics and their associated laboratories

(c) the results are simple to interpret and usually available within ~ 10 minutes. Most of the tests are limited by the fact that they can only detect one species *Plasmodium falciparum*, albeit it is the most life threatening of the malaria species infecting man. Table 8 summarises the available tests.

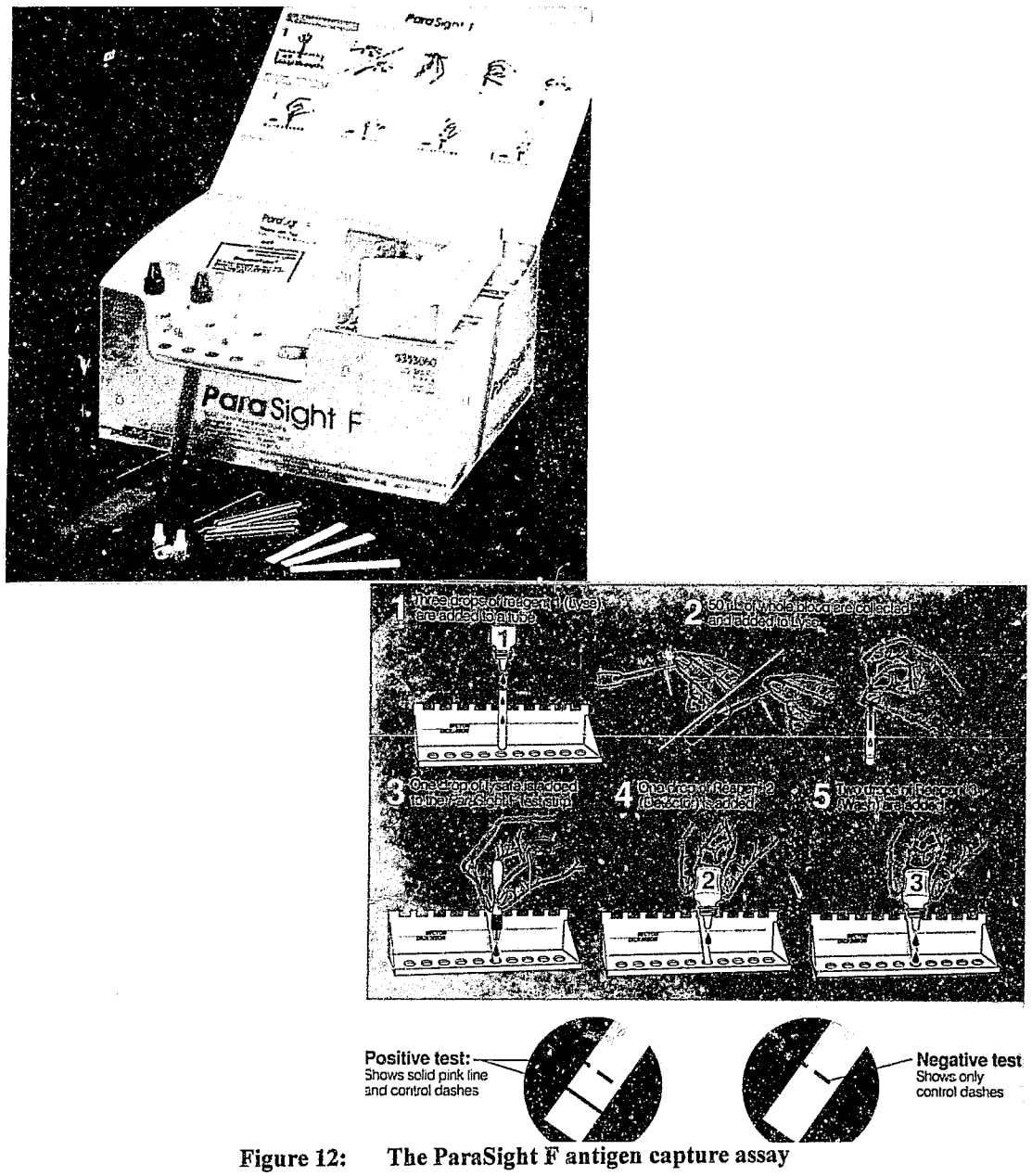
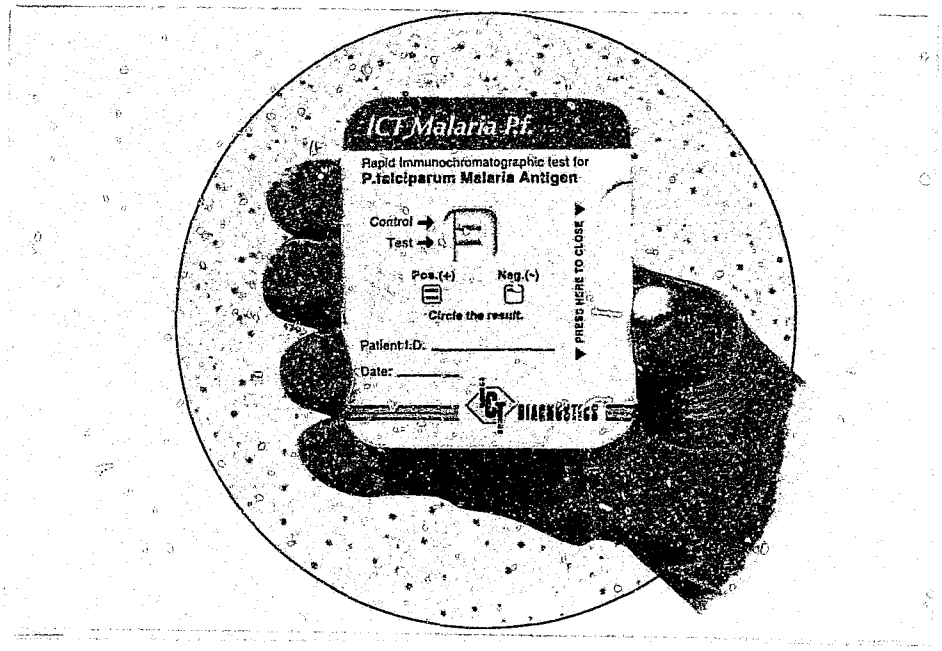
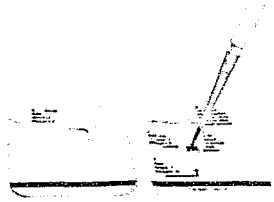


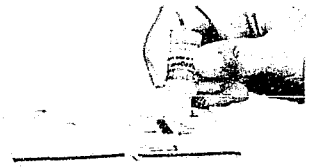
Figure 12: The ParaSight F antigen capture assay



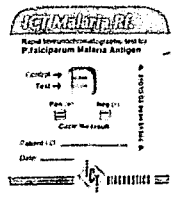
Test procedure



1. Add blood sample



2. Add reagent



3. Close the card and read the results

RESULT INTERPRETATION

Pos.(+)	Neg.(−)	
		Control
		Test

Figure 13: The ICT Malaria P.*f* antigen capture assay

Table 8 : Antigen capture methods used for the detection of malaria

Tradename	Manufacturer	Antigen detected	Sensitivity	Specificity	References
ParaSight ®F	Becton Dickinson (USA)	Histidine-rich protein 2 (*P.falciparum*)	Can detect down to 10 parasites/µl (~94%)	88%-95%	Schiff et al, 1994; Beadle et al, 1994; Ugen et al, 1995; Verlé et al, 1996
ICT ® Malaria Pf	ICT Diagnostics (NSW Australia)	Histidine-rich protein 2 (*P.falciparum*)	~100%	~96%	unpublished data
OptiMAL®	Flow Inc. (USA)	parasite lactate dehydrogenase (pLDH) (is able to differentiate between falciparum and non-falciparum species based on antigenic differences between the pLDH isoforms)	Can detect 100 -200 parasites/µl	?	no data available

OptiMAL® is not a FDA approved diagnostic test and is manufactured to be used for research and development only.

1.8.6 QBC (qualitative buffy coat)

In this method blood samples are placed in acridine orange coated heparinised capillary tubes, and centrifuged. The area below the buffy coat is then examined in situ under a fluorescent microscope to detect the parasitised cells, the malaria parasite stains with acridine orange (Levine et al, 1990). This test has been found to be as sensitive as the detection of malaria using thick smears, however as certain stages of the parasite cycle, like the late trophozoites and the gametocytes may be missed in the buffy layer of the sample, it is advisable to further determine the presence of parasites by making thin and thick smears in parallel. Although it provides only a rough estimate of infection intensity, it is a test which is easier to teach and interpret than a thick or thin film. The techique is also quicker and does not require any special preparation, drying or staining (Warhurst and Williams, 1996). Disadvantages associated with this test include the initial high cost of equipment and the high cost of consumables.

1.9 THE CHEMOTHERAPY OF MALARIA

The treatment of malaria and the prophylaxis against this infection using chemotherapeutic agents is an important aspect in the fight against malaria. The discovery and the development of these compounds have been topics of discussion in chapters and books on malaria, and it is therefore not possible for me to ellaborate in detail on the various drugs ,their mode of action and their side effects in this dissertation. I have however attempted to cover the commonly used drugs and I have tried to briefly introduce the new antimalarials under development. Drug resistance in malaria (White, 1992; White and Olliaro, 1996), albeit relevant in any malarial discussion will not be featured in this introduction.

1.9.1 Historical overview (see also Section 1.1 Milestones in the History of Malaria)

Malaria is one of the oldest infections, being described in early Egypt ,India and China. It stands to reason that attempts at treatment have been made by our ancestral colleagues. The "Jesuit's Powder" derived from the bark of the Peruvian *Cinchona* "fever tree" in the early 1600's was possibly the first record of the use of "quinine" for the treatment of certain "fevers"(Black et al, 1981; Gilles, 1993)). This new remedy was introduced into Europe by the Spanish priests in the mid 1600's and gained popularity on the European continent with its spectacular cures of "fevers and agues". The active ingredients of the bark, however were only isolated more than 250 years after its first application as a therapeutic agent.

1.9.1.1 Chloroquine - the drug of choice ?

During the second world war, following the Japanese invasion of Pearl Harbour in December 1941, the world's regular supply of quinine (from Indonesia, which was occupied by the Japanese army) was denied to the Allied Forces, who were engaged in campaigns in some of the most malarious areas of the world. This prompted a concentrated move to stimulate production of synthetic antimalarials, which had already been initiated as early as 1939. Among the 16 000 compounds screened the most important was SN - 7618/Resochin (a derivative of 4 aminoquinoline), now known as Chloroquine. Chloroquine received its first human trial in the United States in early 1944 and by 1946 > 5 000 individuals had been studied. The drug was found to manifest very few side effects and it could be produced at a moderate cost (Coatney, 1963).

1.9.1.2 Chloroquine - Mode of action

Chloroquine is a antiprotozoal drug used in the prophylaxis and treatment of malaria. Its mode of action is not fully understood. Several mechanisms have been proposed:

1.9.1.2.1 DNA binding hypothesis

Early studies demonstrated that chloroquine (CQ) acts by inhibiting DNA dependent enzyme synthesis of DNA and RNA, and produces rapid degradation of ribosomes and rRNA. The drug was thought to act by intercalating between the strands of the double stranded DNA to form a complex. It was postulated that this complex inhibited the actions catalysed by DNA and RNA polymerase, and deoxyribonuclease (Allison et al, Ciak and Hahn, 1966; Cohen and Yielding, 1965). This theory has been dismissed.

1.9.1.2.2 Ferriprotoporphyrin IX (FP IX) hypothesis

Ginsburg and Warshurst proposed that CQ accumulates in the acid food vacuoles of the intraerythrocytic stage of the malarial parasite and by inhibiting haem polymerase disrupts the conversion of haemoglobin-bound haem into haemozoin (Ginsburg, 1988; Warshurst, 1988). The unconverted haem in the form of ferriprotoporphyrin IX (FP IX) appears to be toxic to malarial proteases and thus further degradation of haemoglobin is blocked, "starving" the intracellular parasite. At certain concentrations FP IX is directly toxic to the cell and to the parasite, and can cause lysis. CQ demonstrates high affinity binding to FP IX , which seems to interfere with its (FP IX) removal. The drug also forms a "lytic" complex which appears to alter the permeability of the cell membrane by interfering with membrane bound proton pumps (Chou et al, 1980; Fitch et al, 1983; Surolia and Padmanaban, 1991).

1.9.1.2.3 Weak Base hypothesis

The intracellular accumulation of CQ results in alkalinization of the acid vesicles within the parasite. This has two possible effects:

(i) A number of malarial proteases mentioned earlier, are most effective at an acidic pH, consistent with their presence in the food vesicles. The raised pH interferes with their action.

(ii) The raised pH results in an increase of the intracellular osmolarity and swelling of the organism.

The use of CQ as a chemotherapeutic agent against malaria has become increasingly hampered by the emergence and spread of resistant *P.falciparum* strains (Cowman, 1991; Alene and Bennett, 1996). Resistance appears to have emerged in the late 1950's and has rapidly spread to all regions of the world where malaria is endemic. In southern Africa the countries affected the most are Malawi and within South Africa, Kwazulu-Natal.

1.9.2 Rationale of Malaria Chemotherapy

The available antimalarial drugs have a well defined range of action against different species of malaria parasite as well as against the different stages of development of the plasmodia within man and the mosquito. Tables 9 a-e summarise the commonly used chemotherapeutic agents.

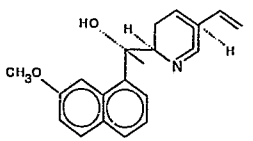
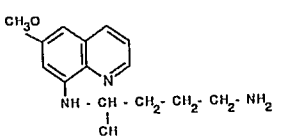
The development of antimalarials is costly and time consuming, however with the emergence of malaria strains that are resistant to the standard drugs newer and better compounds will be required for the future. At this point only a few drugs are at the stage of preclinical or clinical development. These include artemisinin and related compounds

(used by Chinese scientists for over 2000 years), new antifolate combinations, a new 8-aminoquinoline with more activity and less toxicity than primaquine , iron chelators and the use of novel monoclonal antibodies (Looareesuwan et al, 1995; Olliaro and Trigg, 1995; Cabantchik et al, 1996; Hien et al, 1996; Looareesuwan et al, 1996; Mabeza et al,1996; van Hensbroek et al, 1996a; van Hensbroek et al, 1996b).

1.9.3 Quinine - Why so special after 350 years of use ?

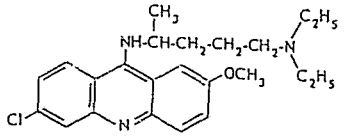
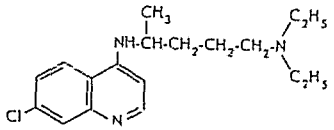
When the use of almost every antimalarial drug is compromised by the development of resistant strains, Quinine "old faithful" is still going strong. There are a few possible explanations why no/limited resistance has emerged to this drug, and these will be discussed in section 5. Quinine has remained effective against *P.falciparum* for 350 years. "This could serve as a lesson to us as we look for ways to preserve the efficacy of our current antimalarials and other drugs" (Meshnick, 1997).

Table 9a: Pharmacology of the commonly used chemotherapeutic agents,against malaria parasites

Drug	Structure	Spectrum of activity	Pharmacokinetics	Tolerance and toxicity	Contraindications
Quinine (Cinchona Alkaloid)	 Quinine (8S,9R)-6'-methoxycinchonan-9-ol	Sporozoites-no action 1° exoerythrocytic stages -no action Asexual forms -fast action Gametocytes - immature (*P.falciparum*) Gametocytes - immature and mature (*P.vivax;ovale; P.malariae*) Latent exoerythrocytic stages -no action No sporontocidal action	Absorbed from the upper intestinal tract. Circulates as a base. Excreted in the urine. t 1/2 ~ 10 hrs	Dizziness,tinnitus tremors,blurred vision. IV - hypotension IMI /subcut- caustic (may lead to fibrosis) oral - bitter taste. General - hypoglycaemia	Caution in patients with renal failure. Caution in pregnancy.
Primaquine* (8-aminoquinoline)	 Primaquine 6-methoxy-8-(4'amino 1'-methylbutylamino)quinoline	Sporozoites - no action 1° exoerythrocytic stages - highly effective against early liver stages Asexual forms - no action Gametocytes - direct and fast acting Latent exoerythrocytic stages - active Active sporontocidal action	Rapidly absorbed from the gastrointestinal tract. Excreted in the urine. t1/2 ~ 6 hrs	Anorexia,nausea,cyanosis epigastric distress,cramps and abdominal pain. Discoloration of urine. Occasionally ~vague chest pain , vomiting and weakness. Haematological: leukopenia, anaemia,methaemoglobinaemia, myelosuppression. Haemolytic anaemia in individuals with G 6 P D	G 6 P D First trimester of pregnancy.

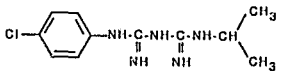
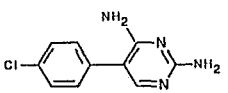
*Weiss et al, 1995

Table 9b: Pharmacology of the commonly used chemotherapeutic agents, against malaria parasites

Drug	Structure	Spectrum of activity	Pharmacokinetics	Tolerance and toxicity	Contraindications
Mepacrine(*) ***(9-aminoacridine)***		Sporozoites - no action 1° exoerythrocytic stages - no action Asexual forms - fast action Gametocytes -as for quinine *Latent exoerythrocytic* stages - no action No sporontocidal action	Rapid absorption from the gastrointestinal tract. High affinity for organs and tissues. Slowly eliminated in the urine	Abdominal cramps, nausea, vomiting and diarrhoea. Dermatitis. Various reversible CNS symptoms.	Syphilis of the CNS. Simultaneous use of mepacrine and the 8-aminoquinolines.
Chloroquine ***(4-aminoquinoline)***		As above	Rapid absorption from the *gastrointestinal tract.* High affinity for tissues especially cells of the liver parenchyma, and melanin containing organs. Slowly eliminated from the tissues. Excreted in the faeces (10%), and in the urine (~60%).	Headache,pruritus and blurring of vision (with high doses). Ocular damage Skin lesions ~ pigmentation problems. IV - hypotension oral - *bitter taste*	Collagen disease

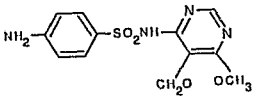
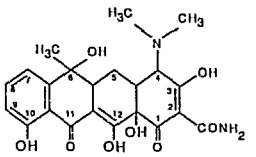
(*) - Mepacrine should no longer be used for the prevention or treatment of malaria as safer and more effective drugs are available.

Table 9c: Pharmacology of the commonly used chemotherapeutic agents , against malaria parasites

Drug	Structure	Spectrum of activity	Pharmacokinetics	Tolerance and toxicity	Contraindications
Proguanil (Biguanide)	 Proguanil N'(p-chlorophenyl)-N-iso-propyldiguanide	Sporozoites - no action 1° exoerythrocytic stages - highly active Asexual forms - active but slow action Gametocytes - no action Latent exoerythrocytic stages - no action Highly active sporontocidal action	Rapid absorption and slow elimination mainly in the urine. 40% is excreted in urine.. Converted to an active metabolite.	Abdominal discomfort, loss of appetite,vomiting and diarrhoea.	None known
Pyrimethamine(*) (Diamino-pyrimidine)	 Pyrimethamine 2,4-diamino-6-p-chlorophenyl-6-ethypyrimidine	Sporozoites - no action 1° exoerythrocytic stages - as proguanil Asexual forms -as proguanil Gametocytes - no action Latent exoerythrocytic stages - some action on *P.vivax* No evident sporontocidal action	Slowly absorbed from the gastrointestinal tract. Binds to tissues and body fluids 20-30% excreted in the urine	Long term use may cause megaloblastic anaemia	Pregnacy(although teratogenic abnormalities have not been reported if used in the prophylactic doses)

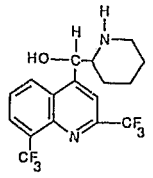
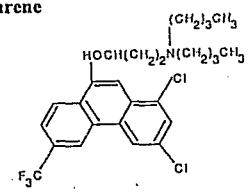
(*) Pyrimethamine is used mainly in the prophylaxis against malaria ; it is a dihydrofolate reductase inhibitor.
Other dihydrofolate reductase inhibitors include proguanil/cycloguanil and trimethoprim.

Table 9d: Pharmacology of the commonly used chemotherapeutic agents, against malaria parasites

Drug	Structure	Spectrum of activity	Pharmacokinetics	Tolerance and toxicity	Contraindications
Sulphonamides and Sulphones(*)	 Sulfadoxine N'-(5,6-dimethoxy-4-pyrimidinyl)-sulfanilamide	Sporozoites - no action 1° exoerythrocytic stages - no action Asexual forms - highly active against *P.falciparum* Gametocytes - given alone they increase production of ? non infective forms(♣) Latent exoerythrocytic stages - no action No evidence of sporontocidal action	Variable rates of absorption and excretion among the various drugs, following the oral route. Excreted mainly in urine t 1/2 ~ 100-200 hrs (#)	Skin reactions like urticaria. Haematological: mild granulocytopenias - agranulocytosis. Haemolytic anaemia in G 6 P D individuals. Methaemoglobinaemia.	Hypersensitivity Premature or newborn infants -first month of life. Use cautiously in hereditary enzyme deficiencies.
Tetracyclines		Sporozoites - unknown 1° exoerythrocytic stages - active against *P.falciparum* Asexual forms - highly active, especially in strains resistant to other standard drugs. Gametocytes - no action Latent exoerythrocytic stages - no action No evident sporontocidal activity.	Most are of low solubility. They are absorbed throughout the gastrointestinal tract but poorly. Excreted in bile and in the urine.	Nausea,vomiting and diarrhoea. Teeth discoloration. Affects bone growth in developing foetuses and young infant. Enamel hypoplasia. May cause hepatotoxicity in pregnancy.	Hypersensitivity Renal impairment. Pregnancy

(*) Sulphonamides and Sulphones can potentiate the effect of pyrimethamine if used in combination with this drug. The commonly used drugs are sulphadoxine and dapsone. (#) t 1/2 of sulphadoxine. (♣) (Puta and Manyando. 1997)

Table 9e: Pharmacology of the commonly used chemotherapeutic agents, against malaria parasites

Drug	Structure	Spectrum of activity	Pharmacokinetics	Tolerance and toxicity(*)	Contraindications
Mefloquine(•) (4-quinoline methanol)		Sporozoites - no action 1° exoerythrocytic stages - no action Asexual forms - marked action Gametocytes - as quinine Latent exoerythrocytic stages - no action No evidence of sporontocidal action	Absorbed adequately from the gastrointestinal tract. Excreted in the bile. t 1/2 ~ 2 weeks. Duration of action may be prolonged.	CNS disturbances including lightheadedness, gastrointestinal distress occasional skin rashes,and rarely phototoxicity. Other CNS effects include: psychosis,depression and anxiety.	Use cautiously in divers and individuals operating dangerous equipment, pilots and long distance drivers.
Halofantrine(♦) (Phenanthrene methanol)		Sporozoites - no data found 1°exoerythrocytic stages - no data found Asexual forms - rapid action Gametocytes - no data found Latent exoerythrocytic stages- no data found Sporontocidal action -no data found.	Fairly rapid absorption with peak blood levels reached within 3-6hrs. t 1/2 ~2.6 days	Gastrointestinal distress including nausea and vomiting, occasional diarrhoea. Neurological disturbances like headaches, tinnitus. Skin manifestations are rare - rash,itchiness. Mild elevation of liver enzymes. ? Cardiotoxicity	

(•) (White, 1994; Jacobs et al, 1987)
(*) (ter Kuile et al, 1995)
(♦) (Restrepo et al, 1996; Nosten et al, 1993; Rinehart et al, 1976; Cosgriff et al, 1982)
These tables (a-e) have been adapted from Black et al, in Bruce-Chwatts *Chemotherapy of Malaria.* Second Edition. Geneva: World Health Organisation, p 56-91

2.0 AIMS AND OBJECTIVES OF THE STUDY

1. The study is structured into 2 major parts:

Part I - the retrospective study

To review the hospital records for all the malaria cases that presented to the Chris Hani/Baragwanath Hospital between January and March 1994, inclusive.

The patients were assessed socioeconomically , clinically, and haematologically.

Part II - the prospective study

To characterise all the patients with malaria that presented to the Chris Hani/Baragwanath Hospital and/or its surrounding clinics between January and March 1995 (inclusive) according to the social, clinical, haematological and immunological aspects of the disease.

2. To provide some insight into the high incidence of malaria at the Chris Hani /Baragwanath Hospital.

3. To assess whether the entity of 'bus stop'/ 'taxi rank' malaria exists ,and whether the climatic conditions in the suburbs surrounding the hospital and the clinics are permissive for transmission of malaria, by infected mosquitoes.

4. To propose some recommendations for the education of our affected population groups, with regards the risks of malarial infection and , the important prevention strategies.

3.0 MATERIALS AND METHODS

3.1 MATERIALS AND METHODS - Part I (Retrospective Study)

3.1.1 General Considerations

The clinical data, including the relevant history at presentation, for this part of the study was collected by reviewing the hospital/medical records of all the patients that were diagnosed with malaria at the Chris Hani/Baragwanath Hospital during January 1994 to March 1994. The hospital records were in the form of (a) the admission and inpatient day -to-day medical file (bed letter), and (b) the outpatient medical record card.

A list of the patients' names and hospital numbers was obtained from the Departments of Haematology and Microbiology of the South African Institute for Medical Research, at the Chris Hani/Bragwanath Hospital where the original diagnosis of malaria was made by routine methodology (i.e examination of thin and thick peripheral blood smears).

Special permission to access these records was granted by the superintendant of the hospital and various administrative personnel in the adult and paediatric departments.

3.1.2 Data collection

3.1.2.1 Social history

The Chris Hani/Baragwanath Hospital serves predominantly the black communities that make up the Soweto area. Other population groups that have access to this hospital include the Indian communities e.g Lenasia ,Lenasia South; the communities of Eldorado Park and very occasionally communities that are located in the Vereeniging area, which are predominantly white. In this study the patients were classified according to their age, sex, socioeconomic status (this was determined mainly by family income)

and whether they were permanent residents of Soweto and its surrounding suburbs,or whether they resided outside South Africa, in which case the noted address may belong to a relative or friend. In most cases a Soweto address will be reflected on the patient's medical records. An alternative residence may be suspected in the event of a travel history documented, but this was difficult to prove on the retrospective data.

3.1.2.2 Clinical aspects

In addition to the age and sex distributions mentioned above , other relevant medical history included information regarding recent or past pregnancies, as well as the pregnancy state of the patient at the time of admission; a previous history of malaria ; particular importance was placed on drug history. Regarding malaria prophylaxis, a record was made whether any drugs were prescribed, by whom they were prescribed , what drugs were given ,at what doses, and for how long. It was also important to note the patients' compliance. Often no record at all was available.

Once the patient was admitted , therapy was initiated in the wards. A list of available drug therapies was made from the recorded data (a number of standard drugs are recommended). The type of drug(s) used , the doses administered and any possible side effects were documented. The duration for which the drug was given was also an important parameter, as this helped determine whether the therapy was optimal.

The presenting symptoms and signs as recorded by the examining doctor at the time of admission were noted. Aspects such as the duration of the symptoms, prior to seeking medical attention, the presence or absence of fever, the presence or absence of organomegaly and especially splenomegaly, the assessment of the cardiovascular and

respiratory systems and careful note was made of any neurological manifestations like loss of consciousness (LOC) and/or seizures.

The outcome of the disease for this admission was documented, and a record was made of any follow up visits to the hospital after discharge.

3.1.2.3 Laboratory investigations

Following admission patients, (in this case those suspected of having malaria or those who presented with a pyrexia of unknown origin [PUO]) had a number of routine blood investigations. These comprised a full blood count (FBC) and differential count, renal function tests (U + E), and malaria smears - thick and thin films. Other optional tests included a random baseline glucose level and liver function tests. The results of these investigations were accessed from the online computer network.

3.2 MATERIALS AND METHODS - Part II (Prospective Study)

3.2.1 General considerations

All patients who were diagnosed with malaria by the Departments of Haematology and Microbiology of the SAIMR at the Chris Hani/Baragwanath Hospital between January 1995 to March 1995, were considered for inclusion in this part of the study. Blood samples for investigation of malaria were received from patients who were already admitted to the hospital wards, from patients that were attending the outpatient facilities like "Med Reg"(a daily outpatient facility that is staffed by medical registrars and consultant physicians) or from patients that were attending the surrounding clinics. The patients from the clinics were usually referred to the hospital, as the staff had been made aware of the study through poster advertisements that had been circulated via the community health services. However, a small number of patients were not sent through for admission especially if the clinical presentation was not urgent; they would receive treatment and be sent home to return to the relevant clinic for follow up. Clinical data from these individuals was difficult to obtain telephonically, and travel to the various clinics was impractical.

A list of the patient's names and hospital numbers was obtained from the Haematology and Microbiology departmental records. An attempt was then made to interview and examine all the patients who presented with malaria to the hospital.

An informed written consent (see Appendix A) was obtained from each patient before inclusion into the study. In the event of a minor, consent was obtained from a parent, guardian, or next-of-kin. The services of an interpretor (usually a nursing sister or a nursing assistant) was often necessary to ensure no misunderstanding and to reassure the

patients that should they wish not to take part in the study , this would not compromise their medical care in anyway.

3.2.2 Data collection

3.2.2.1 Social and clinical aspects

Essentially the same parameters as described in the first part of the study were assessed. The patients were classified according to their age, sex, socioeconomic status , whether they were residents of South Africa or immigrants (this data was often difficult to obtain, as very few individuals volunteered this information, possibly because of their illegal presence in the country). A detailed travel history was obtained, including the country that was visited and the duration of the stay. The duration of the symptoms prior to admission was noted.

A thorough drug history was asked as previously mentioned. Enquiry was made whether medical travel advice had been given, and if so by whom. A history of previous malaria and the treatment that was received was documented.

A detailed history of the presenting symptoms was taken and a full general examination to elicit signs was conducted initially by the attending doctor and then by myself. All the clinical findings were recorded. Important negative signs were also noted.

3.2.2.2 Laboratory investigations

Routine blood investigations included a FBC and differential, U + E, malaria smears, blood glucose and liver function tests. Once the patient agreed to take part in the study and the consent form had been signed, a few additional aspects were considered ,like the antimalarial immunological status of the patient. Blood was taken for immunoglobulin levels and a protein electrophoresis, and serum was sent to the Department of

Parasitology to identify the presence of antimalarial antibodies,via an indirect fluorescence antibody test (IFAT).

3.2.3 Recording of data and results.

The clinical data in the retrospective study was initially recorded on patient information sheets (Appendix B) while the laboratory results were collected as a computer print out/form.

In the second part (prospective study), the patients were given a carefully structured questionnaire to answer (Appendix C), usually with the assistance of an interpretor and myself. The questionnaire addressed all the important aspects regarding the clinical presentation and the history leading up to the admission. Attached to this questionnaire was a clinical information sheet on which the findings of the clinical examinations, and the final outcome of the disease were recorded.

A data base of the clinical and laboratory results was created using Microsoft® Excel (Microsoft® Office '95 for Windows). The clinical results were coded prior to their entry onto the data base. This information was then analysed with the aid of a statistical consultant, Mr John Mansefield (BSc Hons in Mathematical Statistics and Actuarial Science, Wits,1990). The statistical analyses was performed on a standard statistics package, the SAS system, version 6.12 (SAS Institute,Cary,NC) using the BASE and STAT modules.

3.3 METHODS - Specimen Analysis

3.3.1 Haematological assessment

The haematological analysis of peripheral blood samples taken routinely from the patients was performed on a H3 Technicon™ blood analyser (Technicon/Ames, Bayer Diagnostics, Bayer, Evans House Hamilton Close, Basingstoke Hants RG 21 2YE England).

The following parameters were measured:

(a) Hb (this is based on the cyanmethaemoglobin method) - all forms of haemoglobin are readily converted to cyanmethaemoglobin, which is then accurately measured with a photometer at an absorbance of 540nm) - determined in grams per decilitre (g/dl)

(b) HCT (haematocrit or packed cell volume ,PCV) - determined as a ratio

(c) MCV (mean cell volume)- measured in femtolitres (fl)

(d) MCH (mean cell haemoglobin)- measured in picograms (pg)

(e) MCHC (mean cell haemoglobin concentration)- measured in grams per decilitre (g/dl)

(f) WCC (white cell count) - $x\ 10^9$/l

(g) PLTS (platelet count) - $x\ 10^9$/l

3.3.1.1 Reticulocyte counts

These were not requested routinely on every patient. Where the request for this investigation was made, the counts were done manually following the method described by Dacie and Lewis (1995). The results were reflected as a reticulocyte percentage.

3.3.1.2 Differential counts

The method used for the differential count is described in the SAIMR method file under Standard Operating Procedure (SOP) for a Differential Count (Appendix D). The differential counts were done by a haematology technologist, who also commented on any relevant morphological features,including the presence of intracellular parasites. The slides on which the identification of malaria was made, were then referred to myself for microscopy and recording of the findings.

Table 10 lists the normal haematological reference ranges used in the haematology laboratory for adult blacks living on the Witwatersrand (Tickly et al.,1987). The ranges for infants and children are shown on Table 11.

Table 10: Normal haematological reference ranges for adult Blacks living on the Witwatersrand.

PARAMETER	MALES	FEMALES
WCC ($x\ 10^9$/l)	4.0-11.0	4.0-11.0
Hb (g/dl)	14.0-18.0	12.0-16.0
HCT	0.41-0.51	0.37-0.49
RCC ($x\ 10^{12}$/l)	4.43-6.03	4.07-5.13
MCV (fl)	82-100	82-100
MCH (pg)	27-32	27-32
MCHC (g/dl)	32-36	32-36
PLTS ($x\ 10^9$/l)	140-400	140-400
Neutrophils	40-75%	40-75%
Lymphocytes	20-45%	20-45%
Monocytes	2-10%	2-10%
Basophils	0-0.5%	0-0.5%
Eosinophils	1-6%	1-6%

Table 11 : Normal haematological reference ranges for infants and children

AGE	WCC ($x10^9$/l)	Hb (g/dl)	HCT	RBC ($x10^{12}$/l)	MCV (fl)	MCH (pg)	MCHC (g/dl)	PLTS ($x10^9$/l)
Birth	9.0-30.0	18.0-27.0	0.47-0.71	4.8-7.1	109	38	38	140-400
1 week	5.0-21.0	16.0-25.5	0.43-0.68	4.5-6.4	104	36	36	140-400
1 month	5.0-19.5	12.0-21.8	0.34-0.62	3.9-5.9	78	25	33	140-400
6 month	6.0-17.5	10.0-15.0	0.29-0.44	3.9-5.3	78	26	33	140-400
1-2 years	6.0-17.5	10.5-13.7	0.31-0.41	3.4-5.6	79	26	33	140-400
4-5 years	5.5-15.5	11.1-14.7	0.33-0.44	3.9-5.6	83	27	34	140-400
8-13 years	4.5-13.5	10.3-15.5	0.31-0.46	3.8-5.4	85	27	33	140-400

by courtesy: the Department of Haematology, SAIMR, Chris Hani Baragwanath Hospital

3.3.2 Preparation of malaria smears

In this study the identification of malaria was formally made on thick and thin blood smears that were prepared in the Department of Microbiology of the South African Institute for Medical Research at the Chris Hani/Baragwanath Hospital. Occasionally the presence of the malaria parasite was initially detected on the peripheral smear that was prepared for differential counting in the Department of Haematology. After the clinicians were notified an additional sample was usually sent for thick and thin smears to the Microbiology department.

The thick film was used primarily for detection of the parasites (as previously described), and was particularly useful in scanty infections, while the thin film was examined for species identification and for determination of parasite densities. The methods of sample preparation that follow are based on the standard operating procedures for the preparation of blood smears used in the Department of Microbiology:

3.3.2.1 Thick blood smears

3.3.2.1.1 Preparation of thick blood smears

a. Slides are cleaned with alcohol to remove any greasiness that may result in suboptimal smears.

b. A drop of blood is placed in the centre of a slide. The blood is then made into a 10-15mm diameter circle by spiralling from the centre outwards with the corner of another slide.

c. The blood films are air-dried (no heat must be used).

3.3.2.1.2 Staining of thick blood smears

d. The air dried slide is NOT FIXED prior to staining (this allows the water-based stain to lyse the red cells)

e. The stain used is a Giemsa stain (which has been pre-prepared in a phosphate buffer of pH 7.2) . The stain is freshly prepared and filtered regularly.

f. The stain is poured onto the slide and left for 20 minutes.

g. The stain is washed off by flooding the slide with flowing water.

h. Stand the slide on end and allow to air-dry.

3.3.2.1.3 Examination of thick blood smears

a. The thick smear is examined under the microscope using a *x* 100 oil immersion objective.

b. At least 50 fields (or time spent examining the slide ~5 minutes) should be examined before the slide is reported as negative.

The malaria parasites are usually visible in a clear homogeneous layer of cellular debris (see figure 14).

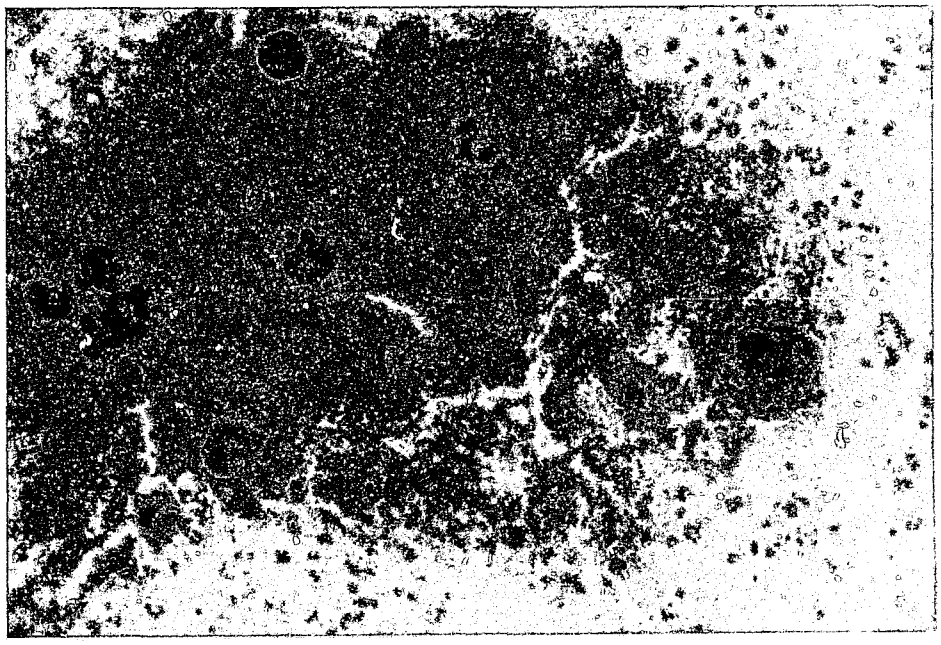


Figure 14: Peripheral blood (*x* 1000) - malaria thick smear

3.3.2.2 Thin blood smears

3.3.2.2.1 Preparation of thin blood smears

a. Slides are cleaned with alcohol to remove any greasiness that may result in a suboptimal smear.

b. A small drop of blood is placed towards the frosted end of the clean slide.

c. A second slide is used as a spreader. Placing this slide at a 30° angle to the first, the blood is streaked in a thin film (slide to slide technique) - see Appendix D pg 2.

d. The slide is allowed to air-dry and then stained.

3.3.2.2.2 Staining of thin blood smears

e. Thin smears are FIXED in methanol for 10 seconds,are removed from the fixative and allowed to air-dry (heat must not be used); fixing the smear allows the red cells to remain intact after staining.

f. A Giemsa stain (as described above) is used to stain the thin smears.

g. The stain is either poured over the slide or the slide is immersed into the stain for 20 minutes.

h. The stain is removed by flooding the slide with flowing water, and the slide is allowed to air-dry .

3.3.2.2.3 Examination of thin blood smears

a. The slide is screened first under low power (*x* 40 dry objective) to identify malaria schizonts and gametocytes.

b. The slide is then examined under *x* 100 oil immersion objective.

c. Up to 150 fields (or time spent on examination of slide ~20-25 minutes) should be examined before the slide is reported as negative.

Thick and thin smears (described above) were prepared directly from a fingerprick or from fresh blood (less than 3 hours old) submitted in a tube anticoagulated with EDTA (ethylenediamine tetra-acetic acid).

3.3.3 Determining the parasite count (parasitaemia/parasite density)

The percentage parasitised red blood cells is generally estimated on the thin blood film. The number of infected cells per 1000 red blood cells is counted, and converted to percent parasitised erythrocytes. Whether the cells contain one or more parasites they are counted as one.

3.3.4 Serological methods - the IFAT

3.3.4.1 Sample collection

Blood for the detection of malaria antibodies was taken from the patient after written consent was given (as discussed in section 3.2.2.2).

Blood was pulled in a red top tube (contains no anti-coagulants or other additives) and allowed to clot. The specimen(s) was then centrifuged in a Beckman GS- 6R series centrifuge (Beckman Instruments Inc.,Palo Alto,California,U.S.A) for 15 minutes at 3000 revs/minute(rpm) to separate the serum from the cellular elements of the blood. The serum was pipetted out and stored in plastic tubes at - 70° C. Each tube was labelled with a number (e.g specimen 1,specimen 2 etc), which corresponded to the patient's name and hospital number. The samples were then batched (batches of 12 samples) and taken to the Department of Parasitology for processing.

3.3.4.2 Sample analysis - the IFAT

Malaria antibodies in patient's serum, especially against *P.falciparum* are detected by a fluorescence - labelled (FITC) antihuman globulin. A fluorescence - equipped microscope is used to read the test.

In my study a commercially available kit (Falciparum - Spot IF ™, bioMerieux,Lyon) was used (see figure 15).

The batched samples were processed by a technologist in the Department of Parasitology of the South African Institute for Medical Research, using standard operating procedures (see Appendix E).

For the purpose of the study, the antihuman globulin used was not a polyvalent conjugate as described in the SOP, but an IgG or IgM specific conjugate.

3.3.4.3 Interpretation of results

The result of the IFAT was interpreted with the assisstance of Dr John Frean of the Department of Parasitology.

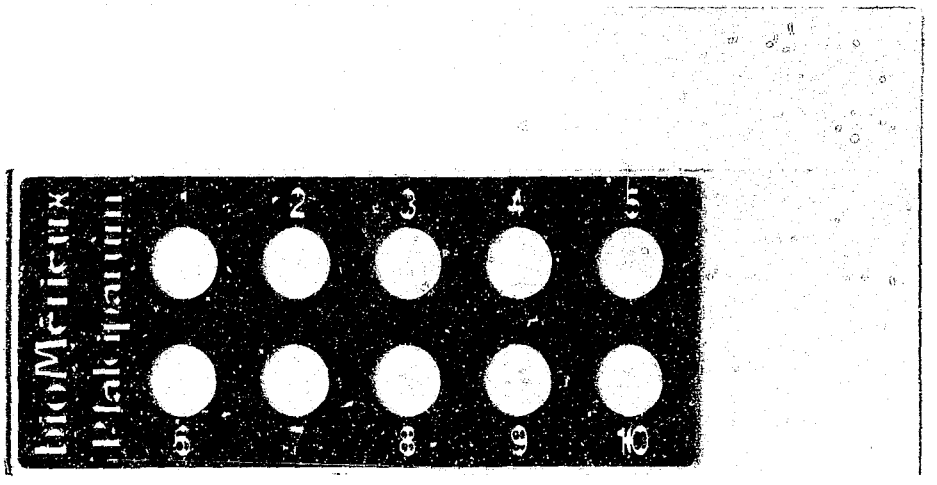


Figure 15: The Indirect Fluorescence Test (IFAT)

The manufacturers of the kit suggest the following interpretation of results:

TITRE	INTERPRETATION
< 1:20	absence of active infection.
1:20 to 1:40	most likely suggests past exposure or possibly active infection caused by a non-falciparum sp.
≥ 1:80	present and active infection.

The results of the tests were recorded initially on a result information sheet .This information was then entered onto the Excel ® data base.

3.3.5 Estimation of immunoglobulin levels

3.3.5.1 Sample collection

Blood for the estimation of serum immunoglobulin levels was taken in a red top tube (containing no anticoagulants or additives) and allowed to clot. The specimens were then centrifuged (as described earlier) and the serum separated from the cellular elements of the blood. The serum was stored at - 70° C until enough samples were collected (batches of 12). Each tube was labelled with a number (e.g specimen1,specimen2) which corresponded to the patient's name and hospital number. The same number that was used for the IFAT was used to identify these specimens.

3.3.5.2 Sample analysis - immunoglobulin levels

The immunoglobulin molecule is Y-shaped and comprises four polypeptide chains (two heavy chains, H ; and two light chains, L) which are linked by disulphide bonds. The H chains (within parenthesis) determine the class of the immunoglobulin. There are 5

immunoglobulin classes: IgA (α), IgG (γ), IgM (μ), IgD (δ), and IgE (ε). There are two L chains - κ and λ. In this study the levels of IgG and IgM were determined.

The specimens were processed by the technologists of the Department of Chemical Pathology of the South African Institute for Medical Research, at the New Johannesburg Hospital. Standard operating procedures for the determinatioon of immunoglobulin levels were used (see Appendix F).

The immunoglobulin that is analysed is detected via an immunoprecipitation reaction that occurs when a specific antibody (test antisera for specific classes i.e anti -IgG or anti -IgM) is brought into contact with the specific antigen. The complexes formed are detected as particles in suspension, through which a beam of light is passed. The intensity of the scattered light is measured by an electronics analyser and the signal is then converted into concentration units.

3.3.5.3 Interpretation of results

The immunoglobulin levels were interpretated by a senior registrar or consultant in the Department of Chemical Pathology. Each result was interpreted in conjunction with a corresponding protein electophoresis , total protein and albumin estimation. Table 12 shows the reference ranges used in this study for the various age groups.

The results of the tests were made available initially on a computer generated hard copy. This information was then entered onto the Excel® data base(Appendix G).

Table 12: Immunoglobulin reference ranges for Black adults and children

AGE RANGE	IgG(g/l)	IgA(g/l)	IgM(g/l)
0-2 weeks	5.3-19.6	0.02-0.12	0.02-0.9
2-4 weeks	4.8-13.4	0.03-0.9	0.3-2.8
4-6 weeks	4.1-15.2	0.05-3.2	0.3-3.8
6-12 weeks	4.1-15.1	0.2-2.1	0.4-3.4
3-6 months	4.2-16.3	0.2-2.3	0.6-2.9
6-9 months	4.8-17.1	0.3-2.0	0.6-2.8
9-12 months	5.3-16.2	0.4-2.0	0.8-2.5
1-2 years	5.6-18.9	0.3-2.7	0.7-3.3
2-3 years	6.4-20.4	0.5-3.3	0.7-3.1
3-6 years	7.4-22.8	0.6-3.4	0.7-3.1
6-9 years	8.4-28.5	0.6-5.5	0.8-3.2
9-12 years	8.9-26.3	1.1-5.1	0.9-3.2
12-15 years	10.1-24.7	1.3-5.0	1.0-2.9
ADULTS	10-20	1.4-4.7	0.5-2.7

by courtesy: the Department of Chemical Pathology, SAIMR, Chris Hani Baragwanath Hospital

3.3.6 MATERIALS - Temperature and rainfall data

The temperature and rainfall data for Johannesburg ,Gauteng was obtained from the Weather Bureau, in Pretoria. Daily rainfall (mm) with monthly totals, and monthly minimum and maximum temperatures for the period 01/01/1994 - 31/12/1996 were received via facsimile. An attempt was made to obtain a similar breakdown of rainfall and temperature data for Maputo,Mozambique, firstly through the Mozambiquan Embassy and Weather Bureau and then by conducting an internet search using Netscape Navigator version 3.0. The information through the above sources was unfortunately not available without a substantial monetary expense.

I was however able to obtain 'historical weather data' for Maputo,Mozambique via the internet through the International Station Meteorlogical Climate Summary, which was

available on the Washington Post web site (WashingtonPost.com). This data is a cumulative summary of high and low temperatures over the last 17 years, and average rainfall measurements for the past 100 years.

3.3.7 Analysis and interpretation of data

The laboratory and clinical data were analysed using frequency tabulations from the information extracted from the data base. A univariate analysis comparing the various haematological parameters, the clinical presentations and outcome of disease was performed using the t-test and the Fisher's exact test. Significance was indicated as a probability $< 5\%$. A logistic regression model was applied to try and determine which variable(s) contributed the most to survival or death.

4.0 RESULTS

The Chris Hani/ Baragwanath Hospital is one of the largest hospitals in the world, and is situated in Soweto,Gauteng. With more than 3000 beds and over 7000 staff, the hospital serves a population of around 3 million people.

During the period of this study there were approximately 100 new adult medical admissions and about 30 new paediatric admissions per day. In addition , the hospital attended to over 300 000 adult outpatient consultations. The paediatric outpatient facility (which operates a 24 hour casualty service) attended to over 48 000 children per year.

During the period of January to March 1994 and January to March 1995, 263 patients (175 male and 88 female) were diagnosed with malaria by the Departments of Haematology and Microbiology of the SAIMR, at the Chris Hani/Baragwanath hospital. Ninety-one of the two hundred and sixty-three patients (35%) were children [< 13 years old]. Figure 16 shows the age distribution of the study patients. The median age was 27 years, ranging from 5 months to 76 years. The mortality rate during my study period was 3% (6/203); clinical data including the outcome of disease was unavailable in 60 cases. Thirty-three of the sixty patients presented to their local community health clinic or outpatient department and were not referred to the hospital for admission; of the remaining 27 cases that were admitted, there was one death prior to the clinical interview, 7 patients were admitted to the short stay ward overnight for observation and confirmation of the diagnosis, and discharged before I was able to interview them and examine them clinically. Within the childhood cases, signed written consent ,for inclusion into the study, from a parent or next of kin was unable to be obtained for 6

patients. Two other parents were unhappy to have their children in the study. Among the adults,3 patients were concerned about their participation in the study and were particularly concerned about the personal details I was collecting,e.g residence, income, nationality .

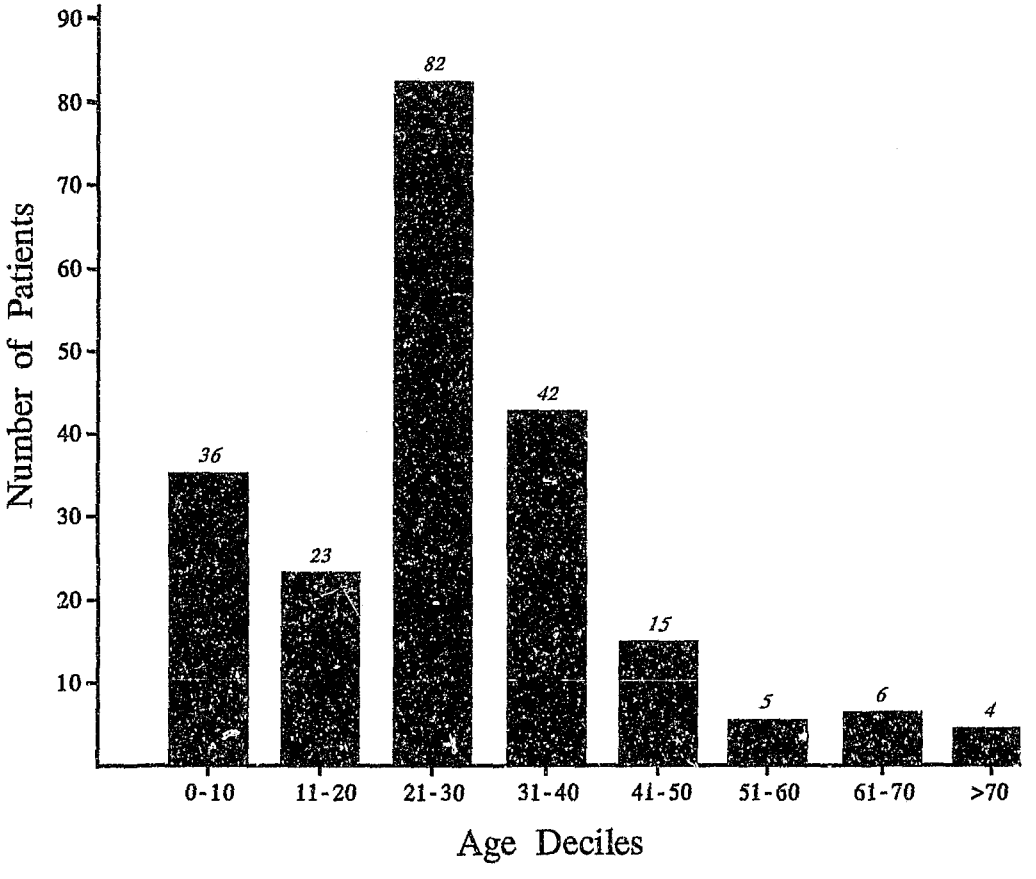


Figure 16: The Age distribution of the study group

These were non English speakers, who appeared to be non-residents of South Africa, and may have been in the country illegally. I was unable to locate 4/60 patients admitted to the hospital, and 4 adult patients were discharged from the hospital before I was able to conduct an interview and clinical examination. There were 15 ICU admissions with five recorded deaths belonging in this group.

The annual incidence of malaria for 1994 and 1995 was 209 and 233 respectively. Figure 17 illustrates the incidence of malaria per month over the years 1994-1996. It is evident from the graph that the number of diagnosed cases are steadily rising from year to year but also from month to month, with the majority of cases presenting between January and May. This indicates a disease prevalence during the summer months. The number of cases for 1996 were included on the graph to demonstrate the persistently increasing trend. The difference between the total incidence per year was shown to be statistically significant ($p < 0.05$). The difference from month to month was highly significant ($p<0.0001$), confirming the seasonal cycle demonstrated on the graph. In addition the incidence of malaria per month was found to be statistically significant across all three years ($p = 0.0131$), with January having the highest incidence of malaria in each year. This data concludes that the number of patients in 1996 (422) appears to have increased significantly over 1994 and 1995.

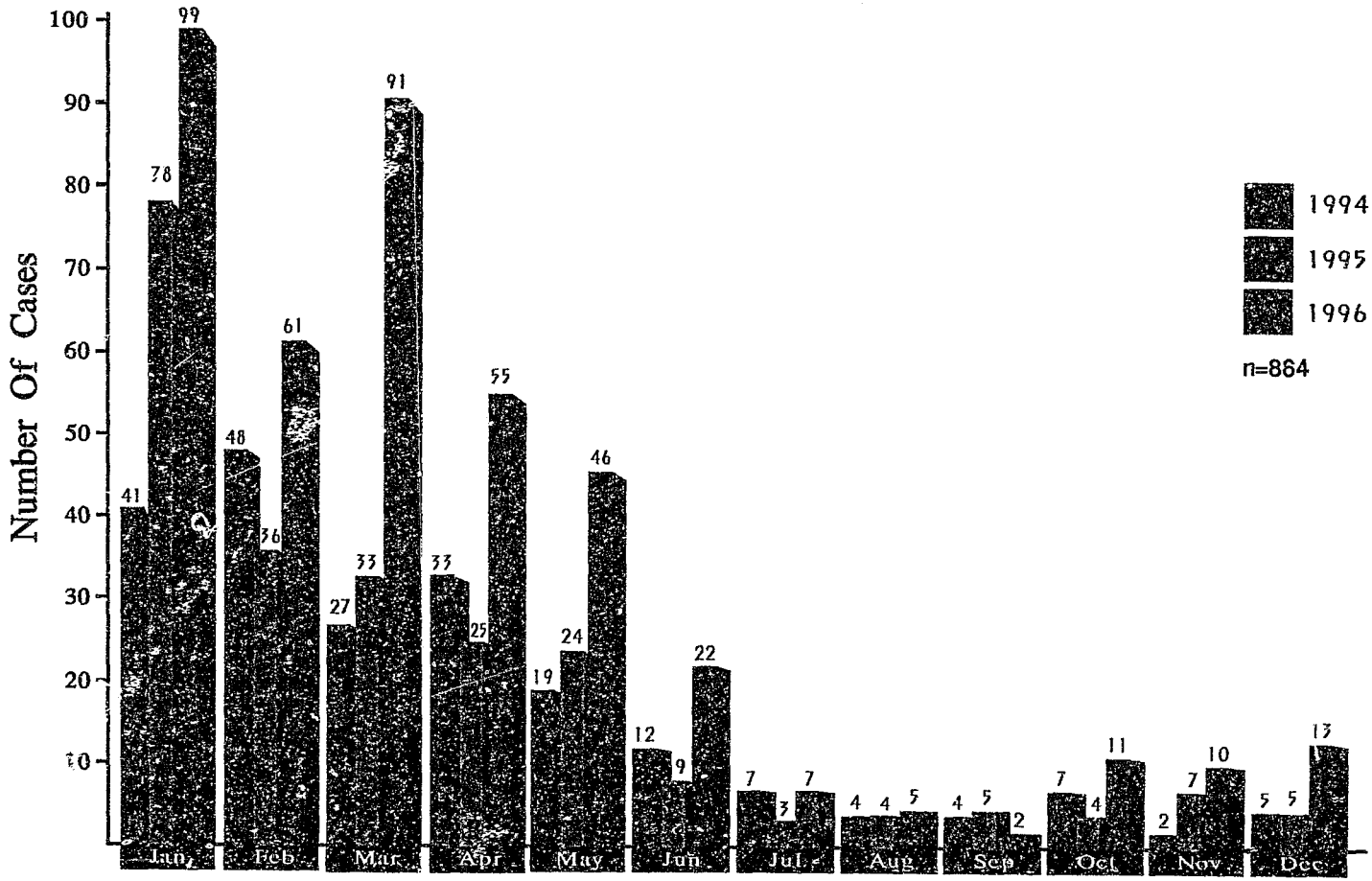


Figure 17 **Monthly incidence of malaria cases at the Chris Hani/Baragwanath Hospital from January 1994 to December 1996**

4.1 SOCIOECONOMIC ANALYSIS

The majority of the patients, 188/203 (93%), that were included in the study were residents of Soweto, with a small proportion of patients ,15/203(~7%), residing in endemic areas, outside and within South Africa (Mozambique- 7; Malawi- 2; Angola- 1; Zaire- 1; Gazankulu- 1; V .. 1; Bophutatswana- 1 and Nelspruit-1). In the present political climate with new socioeconomic classes emerging among the black communities it was difficult to find a generally accepted social classification. If we judge social standards according to the annual family income, 3 major groups were identified with an additional more "affluent" group emerging. Family income was stratified into the following groups based on frequency analysis: a) R 1 000.00 - R 4 999.99 / annum (40%); b) R 5 000.00 - R 9 999.99/ annum (36%); c) R 10 000.00 - R 14 999.99/ annum (18%); d) R 15 000.00 - R 19 999.99 / annum (4%); e) > R 20 000/ annum (2%).

4.2 CLINICAL PARAMETERS

4.2.1 Clinical presentation

Most of the patients presented with a flu-like illness, with the major presenting signs and symptoms listed in Table 13. Fever, the presence of pyrexia (≥ 38°C) on admission ,headache, malaise and joint pain were the most common presenting manifestations of malaria infection. The median duration of symptoms prior to the diagnosis of malaria was seven days (range 1- 21 days).

Table 13: Clinical signs and symptoms at presentation (in order of frequency)

Symptoms and Signs	No.of patients	%
fever	183	70
hyperpyrexia(≥ 38°C)	143	54
headache	134	51
malaise and joint pain	124	47
rigors	104	39
weakness	94	36
splenomegaly	92	35
nightsweats	85	32
hepatomegaly	71	27
jaundice	67	25
abdominal pain	66	25
vomiting	63	24
pallor	61	23
respiratory symptoms	46	17
diarrhoea	34	13
CNS (localising)	17	6
CVS manifestations	12	5
nausea	9	3
CNS (coma)	3	1

More than one sign and symptom was present in most patients

4.2.2 Laboratory data

4.2.2.1 Haematological Parameters

The most common presenting abnormalities included a thrombocytopenia (PLTS < 140 x 10^9/l) in 77% of patients, with a mean platelet count of 103 (± 72.4) x 10^9/l and an anaemia (Hb < 12 g/dl) in 55 % of patients, with a mean haemoglobin value of 11.23 (± 3.2) g/dl (mean values and standard deviations are for both adult and paediatric groups). Severe anaemia (Hb < 5 g/dl) was observed in 13/246 cases (5%). A leucopenia (WCC < 4.0 x 10^9/l) was identified in 48 patients (18%). The mean white cell count was 7.1 (± 3.8) x 10^9/l. Table 14 summarises the haematological data across the children and adult patients.

You will note that haematological results were available on only 246/263 cases. This is because among the community health clinic patients a full blood count was not always requested when a diagnosis of malaria was suspected.

An additional important parameter which is not reflected on the table is the reticulocyte count. This is a fairly good indicator of how active haemopoiesis is, especially erythropoiesis. Only twenty patients (6 adults and 14 children) had a reticulocyte count requested and this is too small a number to provide any meaningful results. Nevertheless, the mean reticulocyte count for both groups was 3.36 (± 5.04) with a range of 0.5 to 18.2.

4.2.2.1.1 Peripheral blood smear morphology

Erythrocytes

A normochromic normocytic anaemia was present in the majority of patients at presentation especially among the adult group. In the paediatric group a more

Table 14: Summary of haematological data for adults and children

ADULT							CHILD					
PARAMETER	n	MIN	MAX	MEAN	MEDIAN	STD	n	MIN	MAX	MEAN	MEDIAN	STD
WCC ($x\ 10^9$/l)	165	1.2	15.5	6.30	5.70	2.569	81	1.9	29.6	8.73	6.80	5.215
RCC ($x\ 10^{12}$/l)	165	0.94	5.82	4.20	4.44	1.085	81	1.0	5.7	3.76	4.01	1.084
HB (g/dl)	165	3.5	17.4	11.8	12.5	3.043	81	2.8	16.0	9.97	10.4	3.186
HCT	165	0.039	0.5	0.358	0.386	0.093	81	0.090	0.78	0.311	0.32	0.109
MCV (fl)	165	67	118.7	86.69	86.6	7.707	81	61.2	103.2	80.79	79.8	7.607
MCH (pg)	165	21.3	38.7	28.4	28.7	2.919	81	18.2	34.3	26.4	26.5	2.798
MCHC (g/dl)	165	30.0	36.0	32.8	32.9	1.241	81	29.6	36.4	32.7	32.8	1.339
PLTS ($x\ 10^9$/l)	165	16	501	91.9	71	67.383	81	14	489	125.8	110	77.372

microcytic picture was noted. The mean MCV was 86.6 (± 7.7) fl and 80.7(± 7.6) fl respectively. The mean MCH for the combined group was 27.7 (± 3.03) pg and the MCHC was 36.4 (± 1.2) g/dl.

Under a x 50 and x 100 oil immersion objective various stages of intraerythrocytic parasites were identified. The distinguishing characteristics were not always obvious e.g Schuffners dots, as the stain used on the peripheral smear is of a more acidic pH. Early ring forms and late trophozoites (Figure 18) were the most commonly seen forms of the *Plasmodium.* The presence of multiply infected red blood cells often suggested a diagnosis of falciparum malaria in the majority of cases. Gametocytes, when present, especially the characteristic banana shape of *P.falciparum* were easily identified within the infected erythrocytes (Figure 19), however it was difficult to distinguish between the

male and female forms. Schizonts were not seen, possibly due to their sequestration from the peripheral circulation.

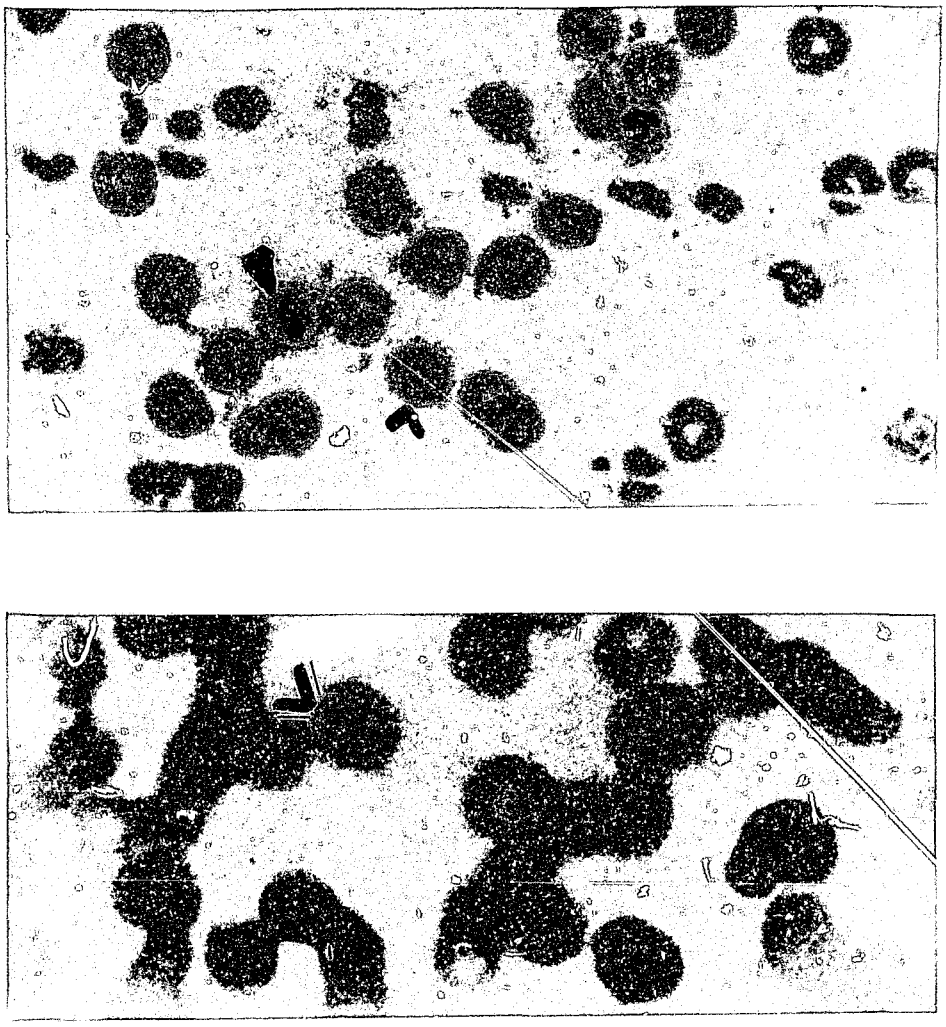


Figure 18: **Peripheral blood smears (x 1000) demonstrating early ring stages and late trophozoites of *P.falciparum*- closed arrow . [Early ring forms in multiply-infected red blood cells-open arrow; applique forms-double arrow]**

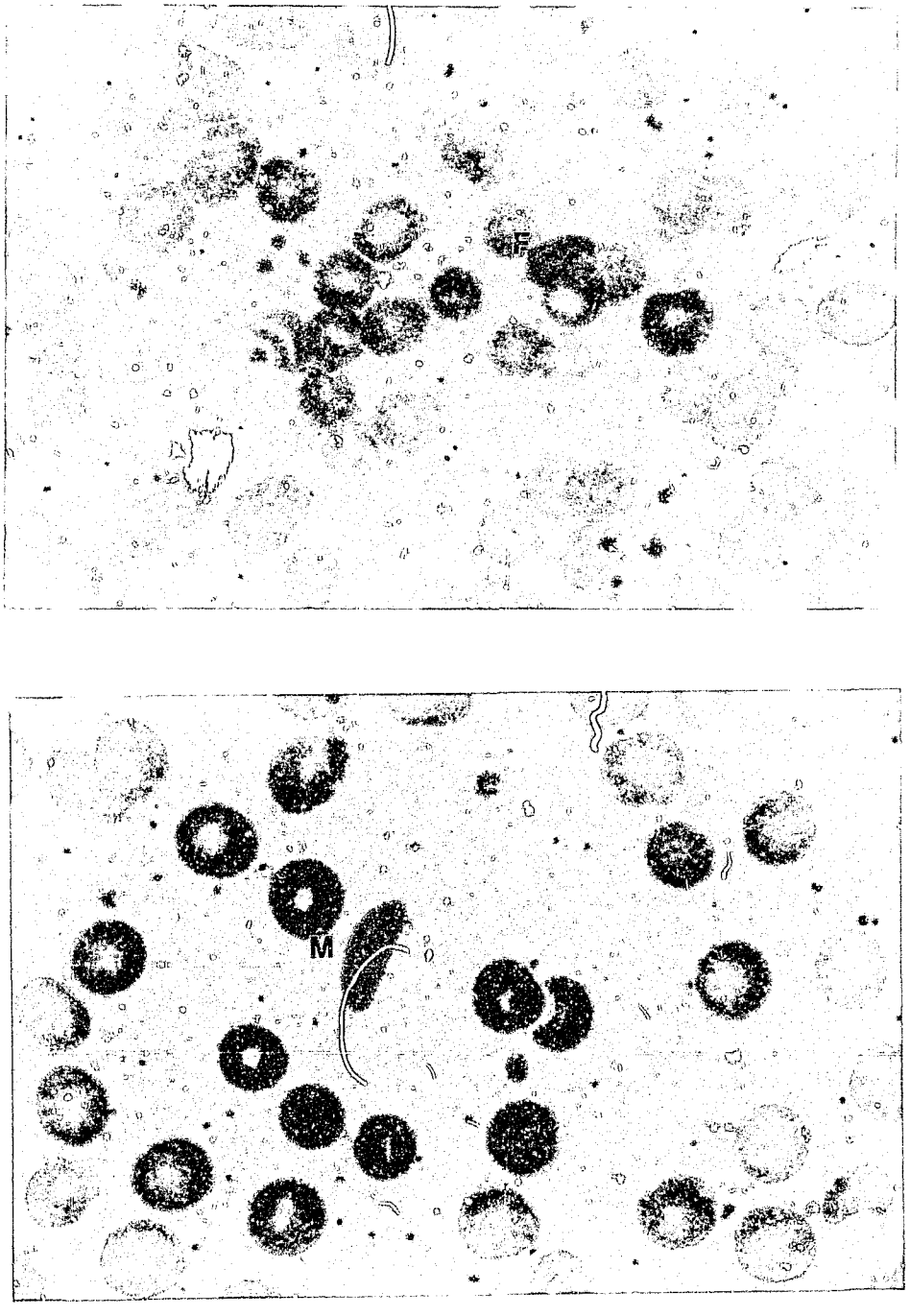


Figure 19: Peripheral blood smears (x1000) demonstrating a male (M) and female (F) gametocyte of *P.falciparum.*

Morphological abnormalities noted in the erythrocytes included the appearance of fragmented cells, irregular membrane outlines (occasional infected cells had a 'burr cell' like appearance), and atypically haemoglobinised cells. Figure 14 (page80) demonstrates the features noted on the thick smear which was used initially as a screening test for the presence of malaria.

Leucocytes

Lymphocytes - Lymphopenia has been reported in patients with malaria (Abdalla, 1990). In the present study 21% of the cases had an absolute lymphocyte count $< 1 \times 10^6/l$. The majority of the patients (67%) had normal lymphocyte counts, with a small proportion (12%) presenting with a lymphocytosis ($> 4 \times 10^6/l$).

A reversed neutrophil lymphocyte ratio was noted predominantly in the paediatric cases. Reactive lymphocytes and plasmacytoid forms (Figure 20 and 21) were often seen on the blood smears. Morphologically atypical lymphocytes however are not specific for malaria infection.

Granulocytes - A neutrophilia was commonly seen in the peripheral blood smears of both the adult and paediatric patients. Morphologically the neutrophils appeared left shifted (hypolobated) and occasional forms appeared vacuolated. Malaria pigment (haemazoin) was readily identified within some cells.

Monocytes - Peripheral blood monocyte numbers were increased in most of the patients with counts ranging between $0.3 \times 10^6/l$ and $2 \times 10^6/l$. The cells appeared large with abundant vacuolated cytoplasm (Figure 22). Monocytes were noted readily engulfing malaria pigment (Figure 23) and occasionally erythrocytes (infected and non-infected).

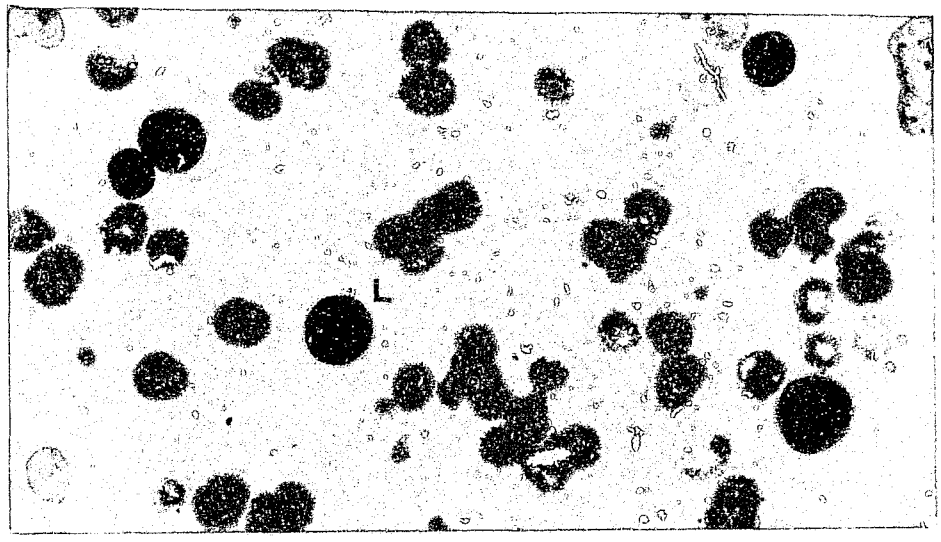


Figure 20: A peripheral blood smear (x 1000) demonstrating reactive lymphocytes (L).

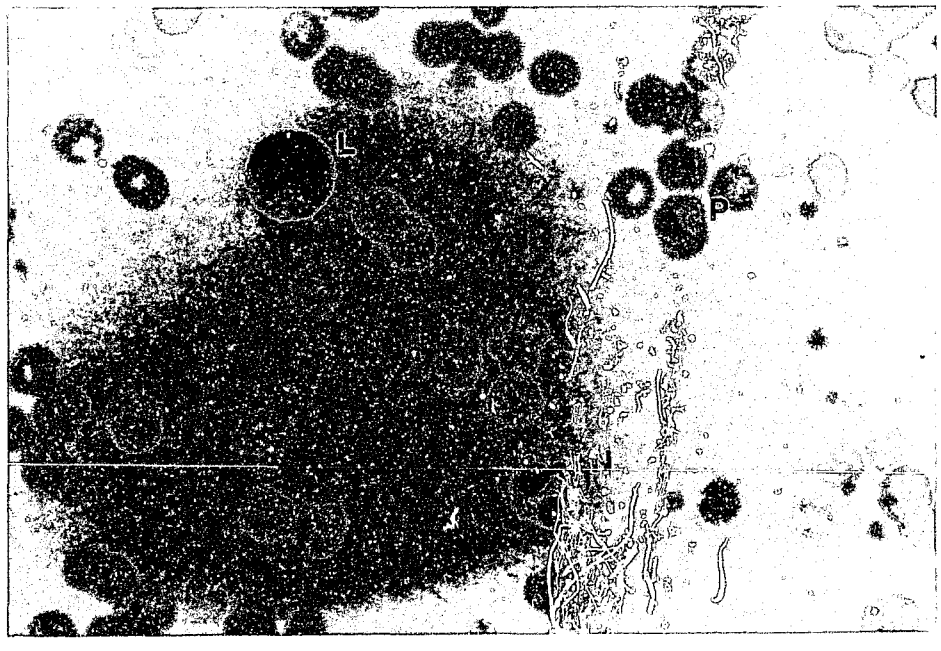


Figure 21: Peripheral blood smear (x 1000) demonstrating an atypical plasmacytoid lymphocyte (L).The background shows a disordered neutrophil (N) and parasitised red blood cells (P).

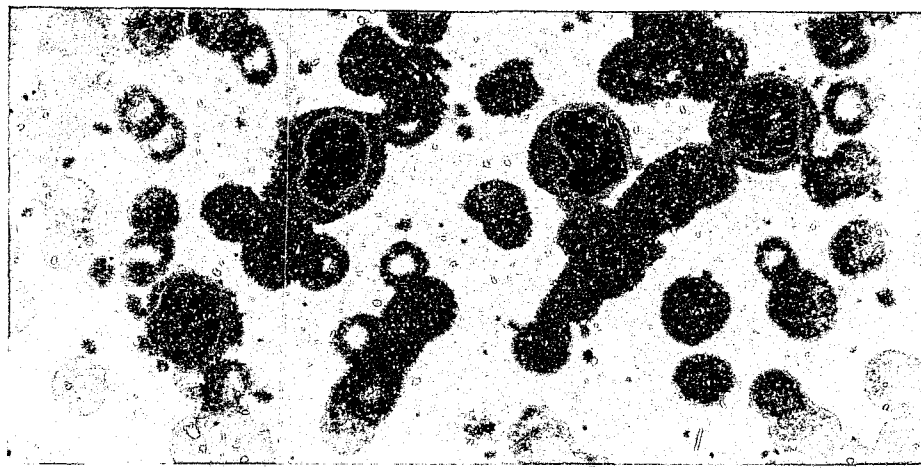


Figure 22: Peripheral blood smear (x 1000) illustrating the various monocytic forms noted in malaria

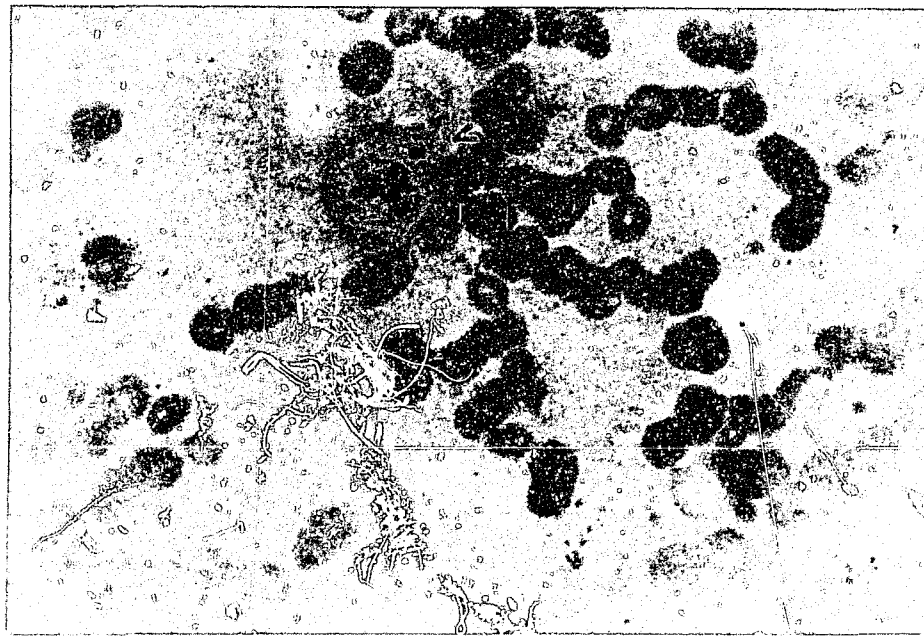


Figure 23: A peripheral blood smear (x 1000) illustrating the phagocytic properties of monocytes, here engulfing haemozoin (arrow).

Other granulocytes - Commonly malaria is associated with an eosinopenia, which was evident in this study. One patient however presented with increased eosinophils (absolute count 0.9 x 10^6/l) - other causes of an eosinophilia were excluded in this patient.

Platelets

Overall platelet numbers were reduced on the blood film, as mentioned earlier. The thrombocytes appeared morphologially normal. Occasional giant platelets were seen, especially in patients who presented with a severe thrombocytopenia.

4.2.2.1.2 Bone Marrow Features

Two patients, were unfortunately subjected to a bone marrow aspirate and trephine procedure to investigate an unexplained thrombocytopenia. The malaria smears in these cases were initially reported as negative. The morphological and numeric features described above were essentially reflected in the bone marrow aspirates. The main changes however where noted in the erythroid series. Erythropoiesis was noted to be fairly active and one of the patients had an erythroid hyperplasia. Various degrees of dyserythropoiesis was noted e.g cytoplasmic basophilic stippling, fragmentation and/or multinuclear normoblasts and sideroblastic changes (Figures 24 and 25).

4.2.2.2 Malaria data

All 263 patients had malaria thick and thin smears for the quantitation of the parasitaemia and for species identification.

The most common infecting species was *P.falciparum* which occurred in 240 patients (91.3%). Ten patients (3.8%) had mixed infections (falciparum: vivax - 7; falciparum: ovale- 3); four patients were infected with *P.vivax* (1.5%), six with *P.ovale* (2.3%),

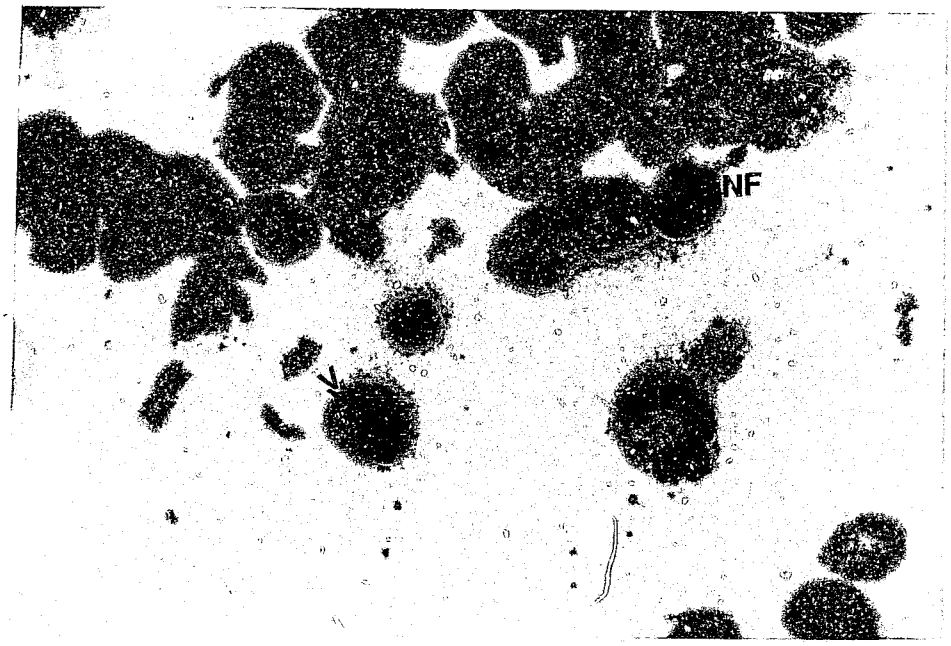


Figure 24: Bone marrow smear (*x* 1000) showing dyserythropoietic features.[Punctate basophilia-arrow; nuclear fragmentation (NF).

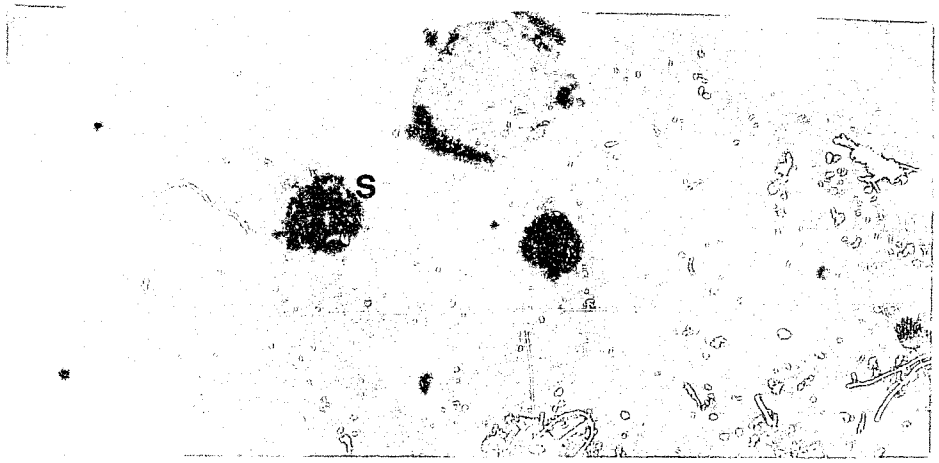


Figure 25: A bone marrow smear (*x* 1000) showing sideroblastic changes within erythroid precursors. [ringed sideroblast (S)].

one with *P.malariae* (<1%) and in two patients the type of infection was not identified (0.8%). Table 15 lists the regions were malaria was thought to have been contracted. In four patients a travel history was not identified. In one family (five patients) the exact region where the infection was contracted was difficult to determine, as they had been travelling through many endemic countries in Africa, however the most likely region has been noted (the family were stranded in Botswana for one week after their vehicle broke down; during this time their malaria prophylaxis finished).

Table 15: Regions where malaria was contracted

Region	No. of patients (n=184)	%
Mozambique (Maputo)	109	58
Swaziland	16	9
Malawi	15	8
Northern Province	10	5
Botswana	7	4
Zambia	7	4
Zimbabwe	7	4
Kwazulu-Natal	5	3
Mpumalanga	5	3
Kenya	1	<1
Nigeria	1	<1
Uganda	1	<1

Among the patients where a travel history was identified, 32/184 had no available data regarding chemoprophylaxis against malaria; these individuals were mainly from the retrospective analysis. Twelve patients (8%) admitted to taking some kind of prophylaxis (Table 16). The remainder of the patients studied i.e 140/184 (76%) had used no prophylactic agents. When questioned why they had not taken any medication prior to their travel, the most frequent response was that they were not aware that any prophylactic medication was necessary. The patients who had taken the prophylactic agents were informed to take them either by a friend (two patients) who had visited an endemic area, had sought medical advice (the family of five), were frequent visitors (three patients) to an endemic region due to their type of employment e.g driver, or had previously contracted malaria while not on any prophylaxis (two patients).

Of the twelve patients who had taken prophylactic agents, five had an insufficient supply of medication, only seven patients had used the correct dosage, and none continued to take the medication upon returning from the endemic area. The most common side effects of the drugs were abdominal discomfort and diarrhoea, and the unpleasant taste of the tablets.

Table 16: Drugs taken for prophylaxis

Drugs	No. of patients (%)
Chloroquine	5 (42)
Chloroquine and Proguanil	1 (8)
Pyrimethamine and Dapsone (Maloprim)	5 (42)
Pyrimethamine and Sulphadoxine (Fansidar)	1 (8)

24/203 (~12%) of the patients gave a history of previous admissions for malaria. One individual had contracted malaria eleven times. This patient is employed in a trucking

company and frequently takes Fansidar as a form of prophylaxis. On his latest trip however, he did not have enough time to organise the tablets before his departure. It is important to note at this stage that Fansidar is not recommended routinely as a prophylactic agent.

Based on a frequency analysis, the levels of parasitaemia were divided into three main groups; < 1% , 1-5% and > 5%. A level of > 5% is defined as hyperparasitaemia according to the World Health Organisation (Warrell et al., 1990). One hundered and thirteen patients (43%) presented with a parasite density of < 1%, 116 (44%) had a parasitaemia beween 1-5% and 34 (13%) presented with a hyperparasitaemia. Table 17 shows the parasite prevalence among adults and children.

Table 17: Parasitaemia frequencies for adult and paediatric patients

Parasitaemia	Adult (%)	Child (%)	Total (%)
< 1%	71 (27)	42 (16)	113 (43)
1-5%	76 (29)	40 (15)	116 (44)
> 5%	25 (10)	9 (3)	34 (13)
Total no. of patients	172	91	263

The median parasite density was 1 (± 4.9) % with a range of < 1% to 36 %. The average time for parasite clearance from the blood after initiation of therapy was 4 days (range 1 to 14 days).

4.2.2.3 Chemistry Results

A number of routine investigations were done on admission. Table 18 summarises the data, and also indicates the number of patients on whom test results are available.

Table 18: Summary of biochemical results

PARAMETER	n	MIN	MAX	MEAN	MEDIAN	STD	N Range
UREA (mmol/l)	218	1	56.6	7.74	5.70	7.46	2.6 - 8.0
CREAT(μmol/l)*	218	13	586	107.8	94	76.9	60 - 120
GLUC (mmol/l)	81	1.7	38	6.62	5.6	5.24	3.0 - 6.0
TP (g/l)	156	30.0	99.0	68.5	69.0	9.16	60 - 85
ALBUMIN (g/l)	156	16.0	48.0	35.7	36.0	5.95	35 - 55
GLOBULIN(g/l)	156	14.0	57.0	32.5	32.0	6.38	23 - 35
TB (μmol/l)	103	6.0	381	44.1	33.0	48.3	4.0 - 21.0
DB (μmol/l)	103	3.0	235	20.1	12.0	29.1	2.0 - 6.0
IB (μmol/l)	103	2.0	146	24.0	18.0	21.3	< 15
AST (U/l)	103	11.0	379	72.5	44.5	73.0	5.0 - 40.0
ALT (U/l)	103	8.0	144	38.7	32.0	28.0	5.0 - 40.0
γ- GT (U/l)	103	7.0	656	47.4	27.0	73.3	0 - 60.0
AlkPHOS (U/l)	103	14.0	316	97.8	80.0	53.6	50 - 130

* Creatinine ranges for Neonates: 27 - 62 μmol/l; Creatinine ranges for children up to 13 years: 44 - 88 μmol/l.

On analysis of the above results, 3% (7/218) of the patients presented with renal failure (creatinine > 265 μmol/l), and approximately 5% (4/81) had hypoglycaemia on admission (glucose < 2.2 mmol/l). Raised bilirubin levels (total bilirubin > 50 μmol/l)

and raised liver enzymes (AST > 40 U/l; ALT > 40 U/l) were recorded in 32% (33/103) of cases. The parameters described above are all indicators of complicated malaria, as defined by the World Health Organisation (Warrell et al, 1990); see also table 4, page 29.

4.2.2.4 Serological results

To assess the immunological status of the patients at the time of diagnosis, blood was taken for IgG and IgM levels and the presence of malaria antibodies against a *P.falciparum* antigen was determined using the IFA test described earlier. These tests were performed only on the cases admitted between January to March 1995, i.e 147 patients. One hundred and one cases were analysed.

The mean IgG and IgM levels were 19.8 (± 6.43) g/l and 2.59 (± 1.08) g/l respectively (IgG range: 7.9 to 44.5 g/l ; IgM range: 0.6 to 5.1 g/l).

The results of the IFA test were difficult to analyse accurately as this test can be subject to human bias. Frequency analysis categorised the results across the various titres i.e ≥ 1:80; > 1:40; = 1:40; > 1:20; = 1:20; and < 1:20 for both IgG and IgM antibodies. In 94% the IgG titre was found to be ≥ 1:80, with 3% of cases demonstrating a negative result (<1:20). The IgM titres were strongly positive (≥ 1:80) in only 65% of the patients tested. In 23% a negative result (<1:20) was documented. A model was then derived which incorporated the interpretation of the various titre combinations according to the manufacturers of the IFAT kit (see section 3.3.4.3). This reflected a positive result in 65 % of patients, with 3% of cases being negative. The remaining 32% were interpretated as positive with either past exposure to malaria or possibly having an active infection with a non-falciparum species. It was difficult to correlate the IgG and IgM titres against the IgG and IgM levels, as the immunoglobulin

levels documented comprised of both specific anti-malaria antibodies and polyclonal antibodies. Specific malaria induced subclasses e.g IgG 1 and IgG 3 were not measured. The most consistent finding, among the patients who had been previously exposed to malaria, was the low parasitaemia at presentation (Table 19). Seventeen of the twenty-four cases presented with a parasite density ≤ 1%. The remaining patients had parasite counts ≥ 2% ,yet did not present with clinically significant disease,except for patient R.K a 30 year old female who had a parasitaemia of 17%. The Indirect Fluorescence Antibody Test did not reveal any differences between the previously exposed and non-exposed groups. Only 6/24 patients had a test result which suggested an underlying protective immune state.

4.2.2.5 Pregnancy data analysis

Among the 88 female admissions, 7 pregnancies were documented. Three were primigravidae, two women presented in their second pregnancy and two were multigravidae. A further two patients presented in the peri-partum period. Of the seven patients, two were discharged with a viable pregnancy and lost to follow up. Among the remaining women, all five pregnancies (2:first trimester; 2:second trimester; 1:unknown) were spontaneously terminated prematurely. Two of the five patients (at 12 weeks and 26 weeks gestation) died from complications of their infection.

4.2.2.5.1 Haematological data

The haematological consequences in pregnancy, especially anaemia and its causes (both physiological and non-physiological), have been well described in the literature (Lampirelli, 1988; Fleming, 1989; Fleming, 1996) and are therefore not ellaborated here any further. The only haematological parameters that were analysed in detail were a haemoglobin level < 5 g/dl (i.e a severe anaemia) and a platelet

Table 19: Malaria data of patients with a history of previous infection

Patient Details			Malaria Data			
Patient	Age	Sex	Parasitaemia (%)	Immunoglobulin Titres		Interpretation
				IgG	IgM	
1994						
G.M.	32	male	<1	N/A	N/A	-
L.M.	33	male	<1	N/A	N/A	-
M.L.	30	male	<1	N/A	N/A	-
M.R.	23	male	1	N/A	N/A	-
M.M.	22	male	<1	N/A	N/A	-
B.N.	19	female	1	N/A	N/A	-
B.M.	55	male	7	N/A	N/A	-
A.P.	34	male	<1	N/A	N/A	-
1995						
E.S.	39	male	8	≥1:80	≥1:80	Present and active infection.
I.M.	9	female	<1	≥1:80	≥1:80	Present and active infection.
F.S.	26	female	1	>1:80	<1:20	Early active infection, possible previous exposure.
G.M.	19	female	2	>1:80	<1:20	Early active infection, possible previous exposure.
S.M.	34	female	<1	≥1:80	≥1:80	Present and active infection.
J.B.	61	male	<1	≥1:80	≥1:80	Present and active infection.
R.S.	59	male	1	≥1:80	≥1:80	Present and active infection.
J.M.	36	male	5	>1:80	=1:20	Early active infection, recent or previous exposure.
N.M.	11	female	<1	≥1:80	≥1:80	Present and active infection.
B.C.	24	male	<1	>1:80	<1:20	Early active infection, possible previous exposure.
R.M.	3	male	<1	>1:80	<1:20	Early active infection, possible previous exposure.
J.M.	42	male	4	>1:80	=1:20	Early active infection, recent or previous exposure.
R.K.	30	female	17	≥1:80	≥1:80	Present and active infection.
H.P.	46	male	3	≥1:80	≥1:80	Present and active infection.
E.M.*	29	male	<1	≥1:80	≥1:80	Present and active infection.
J.N.	20	female	<1	≥1:80	≥1:80	Present and active infection.

* Repeated malaria infections, (11 admissions).

count $< 140 \times 10^9$ /l (i.e a thrombocytopenia). No significant differences were identified between the pregnant and non-pregnant groups for these two variables.

4.2.2.5.2 Chemistry data

The Fisher's Exact test was used to compare the data between the pregnant and non-pregnant groups. Among all the variables tested, pregnancy was shown to be significantly associated only with glucose levels, especially with a glucose level < 2.2 mmol/l (hypoglycaemia), $p < 0.00605$.

4.2.2.5.3 Malaria data

The parasite densities among the pregnant group were as follows: 1 patient presented with a parasitaemia < 1%, 3 patients had a parasite count between 1-5% and 3 women presented with a hyperparasitaemia (> 5%). There was insufficient evidence to show that pregnant women exhibited different levels of parasitaemia than the non-pregnant females ($p = 0.367$).

4.2.2.6 Data analysis of ICU admissions

4.2.2.6.1 Haematological and Chemistry data

15 patients(8F : 5M adults and 1F : 1M child) were admitted to ICU during the study period. A third (5/15) of the patients died (4 adults and 1 child). Among the 4 adults that demised ,2 were pregnant women. Tables 20 and 21 summarise the clinical presentations and laboratory abnormalities within the adult and paediatric age groups. Fever and rigors , pyrexia , jaundice and headache were among the most common presenting signs and symptoms.

Table 20: Clinical signs and symptoms within ICU cases by age group.

(More than one sign and symptom was present in most patients)

	Age Group		
Symptoms and Signs	**Adult (%)**	**Child(%)**	**Total**
fever	9 (67%)	1 (~7%)	10
pyrexia (≥ 38°C)	9	1	10
jaundice	9	1	10
pallor	8 (53%)	1	9
weakness	7 (47%)	1	8
headache	7	1	8
rigors	7	1	8
hepatomegaly	6 (40%)	1	7
CNS (localising)	6	0	6
malaise and joint pain	5 (33%)	1	6
splenomegaly	5	1	6
vomiting	5	0	5
nightsweats	4 (27%)	0	4
diarrhoea	3 (20%)	0	3
respiratory symptoms	2 (13%)	0	2
CVS manifestations	1 (~7%)	0	1
Abdominal pain	1	0	1
CNS(coma)	1	0	1

Table 21: Summary of haematological and chemistry data in the ICU cases by age group.

ADULT						CHILD				
PARAMETER	n	MIN	MAX	MEAN	STD	n	MIN	MAX	MEAN	STD
WCC ($x\ 10^9$/l)	13	1.2	14.8	8.78	4.129	2	8.8	20.4	14.6	8.202
RCC ($x\ 10^{12}$/l)	13	1.15	5.35	3.09	1.110	2	1.75	3.30	2.52	1.096
HB (g/dl)	13	4.5	15.1	8.79	2.92	2	4.2	9.2	6.7	3.535
HCT	13	0.13	0.44	0.265	0.087	2	0.13	0.26	0.195	0.091
MCV (fl)	13	70.9	113	87.9	10.28	2	75.9	78.9	77.4	2.121
MCH (pg)	13	22.4	32	28.6	3.223	2	24.1	27.6	25.85	2.474
MCHC (g/dl)	13	30	35.5	33.1	1.701	2	31.7	35	33.35	2.333
PLTS ($x\ 10^9$/l)	13	21	199	61.9	45.755	2	65	169	117	75.539
UREA(mmol/l)	13	5	49.6	21.89	13.624	1	3.4	3.4	3.4	
CREAT(µmol/l)	13	66	586	276.38	185.818	1	66	66	66	
GLUC(mmol/l)	10	2	37	9.25	10.161	2	3.9	5.8	4.85	1.343
TP (g/l)	13	59	91	69.23	10.191	1	64	64	64	
ALBUMIN(g/l)	13	24	47	33.53	6.995	1	36	36	36	
GLOBULIN(g/l)	13	28	53	35.53	6.887	1	28	28	28	
TB (µmol/l)	13	8	70	46.38	22.137	1	43	43	43	
DB (µmol/l)	13	3	44	22.84	13.483	1	23	23	23	
IB (µmol/l)	13	5	46	23.23	13.000	1	20	20	20	
AST (U/l)	13	20	277	100.692	86.034	1	379	379	379	
ALT (U/l)	13	14	144	42.76	32.853	1	93	93	93	
γGT (U/l)	13	14	189	45.30	46.814	1	23	23	23	
ALKPHOS (U/l)	13	14	246	105.07	59.355	1	163	163	163	

The average haemoglobin across the entire ICU group was 7.74 (± 3.2) g/dl with a mean platelet count of 89 (± 59.6) x 10^9/l. Severe anaemia (Hb < 5 g/dl) was documented in only two of the fifteen cases.

Analysis of the chemistry results indicated that five of the fifteen patients (33%) presented in renal failure (creatinine > 265 µmol/l). 4 patients (27%) had a hypoglycaemia (glucose < 2.2 mmol/l) at presentation and 8 patients (53%) had a documented hyperbilirubinaemia (TB > 50 µmol/l) and raised liver enzymes (AST and ALT > 40 U/l).

The median values have not been included in the tabulation of the laboratory results as these were found to be fairly close to the mean.

4.2.2.6.2 Malaria data

Two thirds of the patients in ICU (67%) had a parasite density of > 5% (hyperparasitaemia). Four patients (26%) had parasite counts between 1-5% and only one patient had a parasite level of < 1%. The median parasite density for the entire group was 9.61 (± 6.14) % (range <1% - 36 %). The average time for the parasites to be cleared from the blood after treatment was 5 days, ranging from 3 to 8 days.

Seven patients had highly positive IgG and IgM antibody titres (≥ 1:80) in keeping with active malarial infection. The mean IgG and IgM levels in the adult patients were 22.2 (± 7.78) g/l and 3.21 (± 1.35) g/l, and in the paediatric case, 15.3 g/l and 1.4 g/l respectively. The infecting species was *P.falciparum* in all 15 patients. Ten of the fifteen patients had a travel history to an endemic area. One patient died before a history could be obtained, and in four cases (retrospective analysis) data regarding travel history was unavailable.

4.2.2.7 Chemotherapy used in the treatment of malaria

Data regarding the drug therapy of malaria was available in 167 patients. The most frequently used drug (in 93% of patients) was quinine , alone or in combination with other drugs (Table 22). The most common route of administration was orally (88% of cases), but a small proportion of patients (12%) received their initial dose of quinine intravenously. Side effects to these drugs were poorly documented in the medical records used for the retrospective analysis. In the prospective arm of the study the only reported complications were that of tinnitus or ringing in the ears in two patients and hypoglycaemia in seven patients. All nine patients were on intravenous quinine at the time. The symptoms settled with reduction of the dose and changing the route of administration. Dextrose saline was administered to overcome the hypoglycaemic effect of the drug. Standard doses were used and the drugs were administered for seven days in the case of quinine and fourteen days for tetracycline/doxycycline; the course was often completed after discharge.

Table 22: Drugs used in the therapy of malaria

Drug	% (of patients)
Quinine alone	48
Quinine and Tetracycline	36
Quinine and Doxycycline	9
Tetracycline alone	2
Chloroquine	4
Primaquine	1

4.2.2.8 Complicated Malaria

Severe anaemia , renal failure, hyperparasitaemia and liver derangements associated with complicated malaria have been discussed under the relevant headings. An additional manifestation of severe malaria that needs to be addressed is cerebral malaria. Regarding the retrospective analysis it was difficult to apply Warrell's (1982) strict definition and I had to rely on the doctor's documented diagnosis. In the prospective analysis the definition was more easily applied. Among the entire study group twenty patients (7%) were diagnosed with probable cerebral malaria, but only 3 patients (1%) could fit the criteria of unrousable coma in the presence of a *P.falciparum* parasitaemia, with no other causes of unconsciousness.

4.2.2.9 Analysis of blood transfusion practise in the malaria patients

Transfusion data was available in 203 cases. Forty-two patients (16%), including the ICU cases, received blood transfusions the main indication being anaemia. Six patients received one unit of packed cells, twenty-six patients were transfused with two units and five patients received more than two units of blood. The patients were grouped into those receiving a transfusion with a HB < 5 g/dl, and those with a HB ≥ 5 g/dl. The age group, patient type, pregnancy state and the outcome of the infection have been summarised in Table 23. Five patients of which three were in ICU, received exchange blood transfusions, the main indication here being a hyperparasitaemia ($> 5\%$).

Table 23: Summary of patients receiving blood transfusions

			ADULT			CHILD	
			FEMALE		MALE	FEMALE	MALE
			Not Pregnant	Pregnant			
Hb	Transfusion	Outcome					
<5	No	Alive	1			3	1
	Yes - 1 unit	Alive	1			1	1
	Yes - 2 units	Alive	1				
	Yes - >2 units	Alive	1				
≥5	No	Alive	31	3	94	13	16
		Dead			1		
	Yes - 1 unit	Alive	1				2
	Yes - 2 units	Alive	2		10	1	4
	Yes - >2 units	Alive			2	1	
		Dead		1			
	Exchange Tx[a]	Alive	1	1	1		1
		Dead		1			

[a] Abbreviation: Tx - transfusion

4.3 UNIVARIATE AND MULTIVARIATE ANALYSIS OF LABORATORY DATA

Categorical and continuous data were analysed using the Fisher's exact test and t- tests in order to determine which of the variables was significantly associated with a poor outcome. Among the categorical data (parasite load, haemoglobin, creatinine and glucose level, and bilirubin levels) only hyperparasitaemia (>5%), p=0.000438 and renal failure (creatinine > 265μmol/l), p=0.00852 were significantly associated with reduced survival. A univariate analysis (Table 24) of the continuous data comparing survival and death identified a significant difference between the WCC (p=0.0281) and the HB (p=0.0084) levels in the survivors versus the patients who died.

Table 24: Univariate analysis comparing survival and death with regard to continuous data (mean ± standard deviations)

	Survivors		Deaths		
Variable	**n**	**Mean ± STD**	**n**	**Mean ± STD**	**p-value**
Age	195	26.8 ± 15.63	6	29.8 ± 12.51	0.6260
WCC	195	7.14 ± 3.84	6	10.7 ± 5.41	0.0281
HB	195	11.3 ± 3.25	6	7.7 ± 2.22	0.0084
PLT	195	99.9 ± 68.82	6	68.6 ± 53.41	0.2725
UREA	181	7.49 ± 6.09	6	18.7 ± 18.61	0.1991
CREAT	181	104.9 ± 66.2	6	238.3 ± 223.0	0.2032
TB	82	44.2 ± 49.3	6	46.0 ± 13.5	0.8310
AST	82	73.7 ± 72.3	6	136.3 ± 123.2	0.2726
ALT	82	40.5 ± 29.8	6	41.5 ± 25.4	0.9380

A logistic regression model was applied to the continuous and categorical data. This showed high parasite loads [p=0.0070; O.R=0.891(95%CI: 0.811 - 0.981)] and raised creatinine levels [p=0.0016; O.R=0.992 (95%CI: 0.987-0.997)] to be significant predictors of poor outcome. The statistical analysis suggested a strong colinearity between these two variables.

4.4 TEMPERATURE AND RAINFALL MEASUREMENTS

Monthly and annual rainfall totals were obtained for the Johannesburg area, Gauteng, for the period January 1994 to December 1996. These are summarised in Figure 26. Minimum and maximum monthly temperatures for the same periods are illustrated in Figure 27.

Similar climatic data was obtained for Maputo, Mozambique (an endemic region) to compare with local weather conditions (a non-endemic region) in the bid to ascertain whether the climate in the Gauteng province is suitable for malaria transmission. The Maputo data (illustrated on the transparencies attached to figures 26 and 27) mainly comprises cumulative values for temperature (collected over a period of more than a decade) and rainfall (data for up to a hundred years). Temperature and rainfall measurements for the Soweto area (Gauteng), unfortunately, were not available.

The annual rainfall, in Johannesburg, across the three years appears to have increased, however the monthly minimum temperatures recorded may not be ideal to permit parasite transmission in the city. The minimum temperature required for the transmission of *P.falciparum* is about 20°C; the highest minimum temperature recorded in Johannesburg was 14.5°C, during the summer months.

Although the average annual precipitation is comparable, there appears to be a less dramatic monthly temperature variation in Maputo. Here the highest minimum temperatures recorded during the summer months average 22°C.

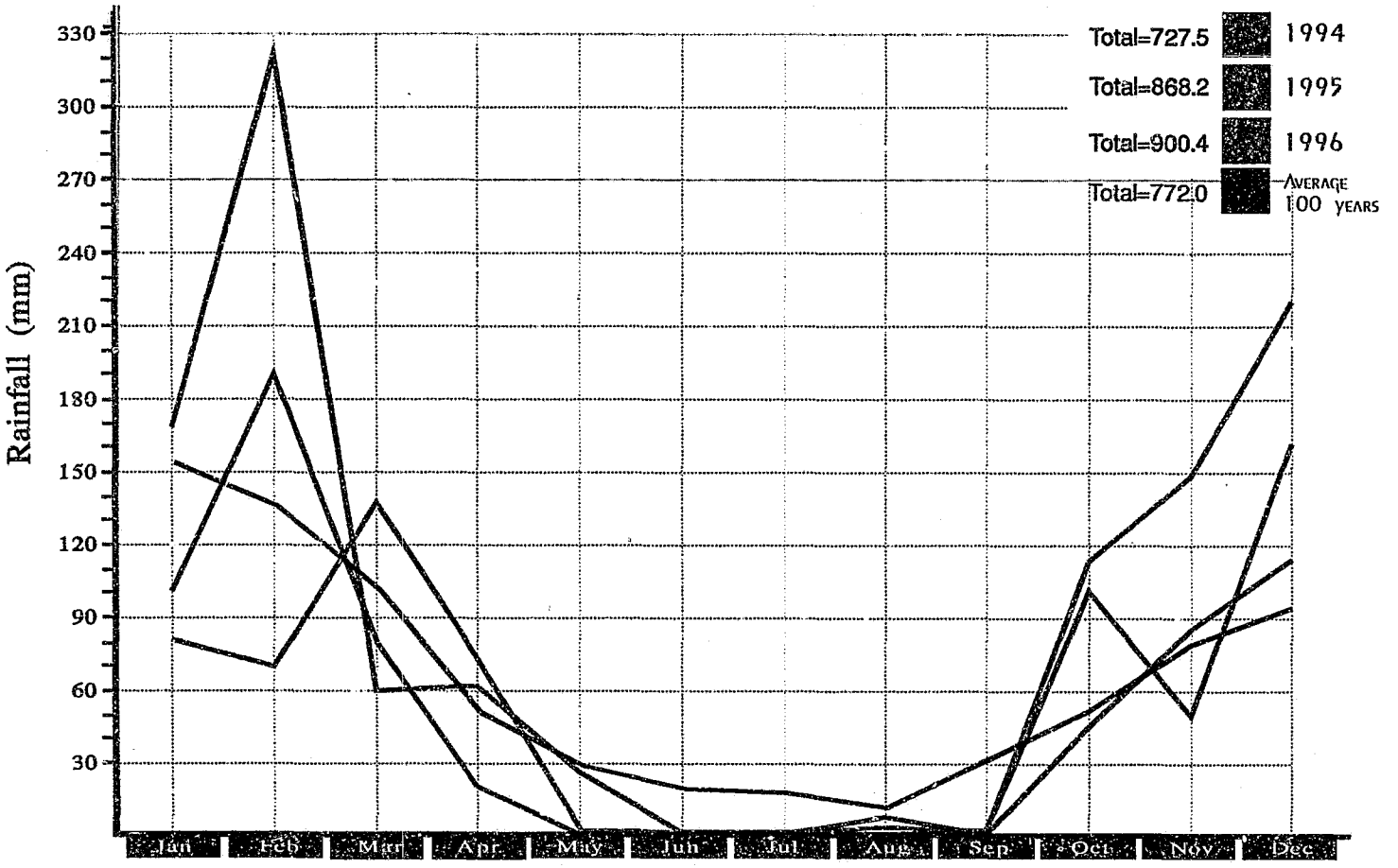


Figure 26 Monthly rainfall (mm) Johannesburg, Gauteng from January 1994 to December 1996
Average rainfall (mm) Maputo, Mozambique from 1894 to 1994 (average 100 years)

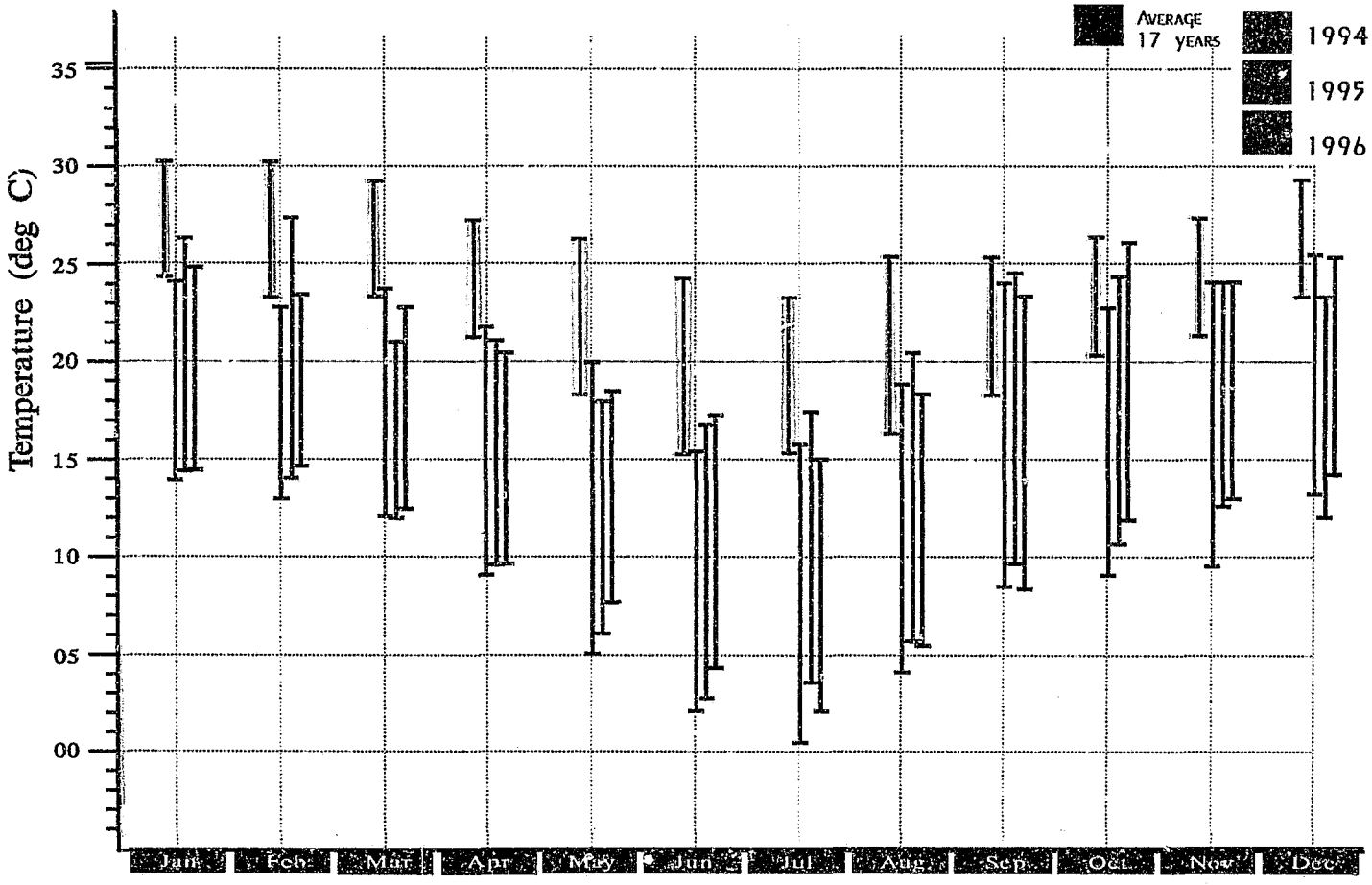


Figure 27 Monthly temperature variation (deg C) Johannesburg, Gauteng from January 1994 to December 1996
Monthly average temperature variation (deg C) M. .puto, Mozambique from 1977 to 1994 (average 17 years)

The differences in the weather patterns between the two regions (particularly the minimum temperatures) alone may be sufficient to prohibit malaria transmission, however there are more factors which need to be considered before any conclusions can be made. One such factor is the mosquito vector (this will be discussed further in section 5.0).

5.0 DISCUSSION

5.1 INCIDENCE OF MALARIA

"South Africa could be facing its worst malaria epidemic,..." This appeared in a leading newspaper in March 1996. At the Chris Hani/Baragwanath Hospital, 422 cases were admitted between January to December 1996, a significant increase over the previous two years. These numbers do not necessarily mean that malaria is on the increase; they may indicate that more people are having access to the hospital, and/or that more patients are being correctly diagnosed. It may also reflect that the number of travellers to malaria areas has increased. In order to accurately conclude whether the incidence of malaria is truly reaching epidemic levels, we would have to present the data of hospital admissions as a proportion of the population infected This was out of the scope of this dissertation. Nevertheless the problem could be exacerbated by the heavy rains, experienced during the summer months, which may leave behind pools of water that may be attractive sites for breeding mosquitoes. Extreme rainfalls on the other hand, may wash away potential breeding sites and interrupt transmission. The environmental conditions reflected in the data were not entirely conducive for malaria transmission in the urban areas. Both the vector and the parasites are poikilotherms, and generally the more elevated the temperature is the more enhanced the rate of development and activity becomes, thereby increasing transmission. The average minimum temperatures ranged from 0.2°C to 14.5 ° C, and the maximum temperatures from 15.1°C to 27.5° C. The wide variation in the temperatures may not be ideal for the development of the mosquito, especially during its aquatic stages (eggs and pupae). There appears to be a relationship between temperature and the rate of development of the vector. At lower

temperatures the vector takes a longer time to develop (Lindsay and Birley, 1996). In addition temperature has also been shown to affect the time it takes for the parasite to become infective in the vector (sporogonic cycle). The warmer the temperature is, the shorter the duration of the sporogonic cycle. The critical minimum temperature for parasite development is uncertain. Values range from 16°C to 19°C for *Plasmodium falciparum*. High temperatures are also important in transmission. Very high temperatures ~35° C are detrimental to the development of the parasite.

From the rainfall and temperature data presented above it appears evident that the mosquito vector and the parasite will not survive the extreme weather conditions that are noted on the 'Highveld' in winter and may not survive during the summer. Global warming however could see malaria transmission pushed up to higher altitudes (Lindsay and Birley, 1996). In endemic areas, global warming has seen an extension of the season of malaria transmission with increased number of cases becoming infected. Even if the conditions were suitable for vector and parasite development, it has been documented that the malaria transmitting *Anopheles* vector does not exist in Gauteng (Coetzee et al, 1993).

In the above study the majority of the patients (>88%) had contracted malaria, which was predominantly of the falciparum type, from a neighbouring country with Mozambique being the most popular travel destination (58%). The seasonal incidence appears to coincide with the Christmas and Easter Holidays. The numbers are particularly high during the January months across the three years. This is due to a suspected high number of Mozambicans becoming ill while travelling between Mozambique and South Africa as migrant workers or, while visiting relatives; and by South Africans visiting Mozambique and returning with malaria. Among the four

patients who did not travel, one patient gave a history of a relative visiting from Maputo. The data presented above appear to suggest that local transmission is unlikely however, transported mosquitoes have been known to transmit infection outside the immediate vicinity of the transporting vehicle. Could this be a case of malaria caused by a 'travelling' mosquito ?

5.2 MALARIA AWARENESS AND PROPHYLAXIS

The single most important issue that became apparent in both the retrospective and prospective arms of the study, was the lack of awareness regarding the infection. Even individuals who gave a history of frequent travel into endemic regions had no basic knowledge of malaria and its consequences. The next most important problem identified was the insufficient use of / poor compliance with, prophylactic agents. Only twelve patients took some form of prophylaxis, and none continued to take the drugs after they returned from the endemic region, mainly because of the unpleasant side effects of the medication. Half of the patients were on a form of medicine that is no longer acceptable for prophylaxis e.g dapsone-pyrimethamine (Maloprim) and sulphadoxine-pyrimethamine (Fansidar), mainly due to the occurrence of serious side effects, but also due to the reported pyrimethamine resistance. These drugs continue to be used in the treatment of acute malaria, and certain health authorities e.g in Zimbabwe still recommend maloprim as a form of prophylaxis (Lombard et al,1991). Generally accepted forms of prophylaxis include CQ alone or in combination with proguanil, mefloquine (Lariam) (the potential side-effects restrict its use in all individuals), and tetracycline or doxycycline (this is particularly useful for travellers to CQR areas). Held et al (1994) identified a few risk factors that may be associated with poor or non-

compliance in the use of drug prophylaxis. Compliance increased with age, and shorter duration of stay. The type of travel and travel destinations also influenced travellers' behaviour i.e tourists on organized tours were more compliant than individual travellers. Travellers to southern Africa were the most compliant, followed by those travelling to East Africa. The compliance rate for those going to West Africa was ~32% and for Central Africa was ~22%. The final point that was addressed regarding prophylactic behaviour was whether the traveller had made use of any information sources before travel. Those who did not were significantly less compliant and often took incorrect drug dosages. In the individuals who made pre-travel enquiries there appeared to be more compliance among those who had made use of more than one source of information. Antimalarial drugs must be taken regularly to be effective. The Department of Health in collaboration with the TPS Drug Information Centre have printed several brochures which are aimed mainly at the medical practitioner who provides the travel advice, but may also be and should be aimed at the traveller. The brochures provide comprehensive information regarding risk areas and provide easy to follow tables with drug information and dosage schedules (Department of Health, 1996; Department of Health, 1994; TPS Drug Information Centre, 1993). These are often available on request free of charge.

5.3 AVAILABILITY OF ANTIMALARIAL DRUGS

It is all very well educating the doctors to give better advice, and to educate the travellers to seek these prophylactic agents, but these should be made more readily available to the populations that are most at risk. At the Chris Hani/Baragwanath

Hospital the repertoire of drugs (that are affordable to the patient who seeks medical attention at this government institution) is limited e.g chloroquine and doxycycline are the only drugs available for prophylactic use, and clearly with the increasing CQR strains emerging , the availabilty of an additional drug like proguanil is indicated. In addition doxycycline is contraindicated in children < 8 years and preferably < 12 years old, because of the complications associated with the tetracyclines e.g enamel hypoplasia, abnormalities in bone growth, and the discoloration of teeth and bones (Black et al, 1981). Sulphadoxine-pyrimethamine (Fansidar) is on code for use but as mentioned earlier it is not a suitable prophylactic agent and is reserved mainly for treatment. Drugs like chloroquine, proguanil and mefloquine are naturally readily available over the counter in non-government pharmacies, but the retail prices are often unaffordable for most of the population that requires them especially since 76% of the individuals in the Soweto area earn < R1000.00 per month (40% earn between R 1000.00 and R 4999.99; 36% earn between R5000.00 and R 9999.99). Often these individuals have more important priorities than the acquisition of antimalarial drugs e.g transport costs and food.

The various chloroquine preparations range between R 25.70 - R 28.83 for up to 20 tablets (chloroquine alone) and when in combination with proguanil the price increases to R 66.10 for a 5 week course (a family of four would require over R 250.00 worth of tablets); proguanil alone retails at ~ R82.00 /100 tablets and mefloquine ~ R102.50 /8 tablets (a minimum of 5 tablets is required, and a weekly dose is recommended for each week in a malarial area). Unless more of these drugs are made available to the government hospitals and the surrounding health clinics, the issue of chemical prophylaxis will not be solved.

5.4 GENERAL PROPHYLACTIC MEASURES

Prophylactic behaviour however, does not end with chemoprophylaxis, and this is not 100% effective as has been demonstrated in the patients on prophylactic agents who develop malaria, albeit less severely. Alternate measures may help reduce the cost of malarial control e.g the use of bed nets (Curtis, 1994; Luxemburger et al, 1994; Van Bortel et al,1996) and insect repellants (Department of Health, 1994; Department of Health, 1996), the avoidance of being outdoors between dusk and dawn where possible, and to wear protective clothing to minimise the risk of excessive contact with the mosquito vector.

5.5 DIAGNOSIS AND TREATMENT

Travellers are at risk of developing malaria for up to 2 months after leaving an endemic area, however cases of malaria have been reported to have developed as late as one year after leaving the malaria area. Delays in diagnosis and subsequently treatment are responsible for the mortality associated with this infection. In this study the median duration of symptoms prior to diagnosis was seven days. The mortality rate was ~3% and deaths were related to complications of the infection e.g renal failure, anaemia and cerebral malaria, rather than a delay in diagnosis and initiation of therapy.

5.5.1 Clinical presentation

The presenting clinical features were consistent with other studies in South Africa (Jairam et al, 1990; Soni and Gouws,1996). Splenomegaly was documented in less patients than usually reported, but this may be an underestimated figure, as I was unable to confirm the clinical presentation of the patients in the retrospective arm of the study.

The prodrome of fatigue, malaise, feverishness and headache was classical in most of the patients (>40%). At presentation few patients gave the classic 'textbook' episodic description for fever (every 48 hours in *P. falciparum* malaria - tertian periodicity). This is an observtion usually made in patients who present with untreated longstanding infection (Warrell, 1993; Murphy and Oldfield, 1996).

The median age of the patients who presented with malaria was 27 years which is consistent with other reports (Sharp et al, 1988; Jairam et al, 1990; Soni and Gouws, 1996), whether this reflects the population structure of the Soweto area is uncertain.

5.5.2 Laboratory analysis

5.5.2.1 Haematological data

Analysis of the haematological data confirmed previously documented findings. In this study severe anaemia was present in 5% of patients. Anaemia is an important cause of morbidity and even mortality. A significant difference was found in the HB levels in the patients who survived versus those that died. The mean HB in the patients that demised (7.7 ± 2.22 g/dl) was significantly lower than that of the survivors (11.3 ± 3.25 g/dl), p=0.0084; but only one patient who died had a HB < 5g/dl i.e a severe anaemia.

The mechanisms of anaemia in malaria are due to two main causes: a) haemolysis and b) inappropriate marrow responses. Haemolysis can arise as a consequence of erythrocytic parasitisation, with the infected red blood cells destroyed at schizogony; there may be an immune mediated component, as some authors have reported a positive Coomb's test in the setting of severe malaria (Abdalla,1990; Newton et al,1997). This however is yet to be confirmed. Erythrophagocytosis and sequestration in the reticuloendothelial system has been described. Together with the PRBC, uninfected erythrocytes may also be

removed in the spleen (Phillips and Pasvol,1992). Finally drug related causes may predispose patients to anaemia. Certain antimalarials like artemisinin induce haemolysis of the PRBC that are parasitised with trophozoite and schizont stages (Orjih, 1996).

Dyserythropoietic changes were found in the marrows of the two patients who were investigated for thrombocytopenia and subsequently diagnosed with malaria. This is not an uncommon finding (Abdalla,1990) and further potentiates the anaemia. The cause of the dyserythropoiesis in malaria is not entirely clear; a few theories have been proposed (Abdalla,1990; Weiss,1990) these include nutritional deficiencies, cytokine production (e.g TNF), direct parasitisation of erythroblasts by malaria parasites (this has only been demonstrated in vitro), and obstruction of the marrow sinusoids by PRBC which leads to marrow hypoxia.

Cell cycle studies using spectrophotometry and thymidine uptake techniques have demonstrated an increased proportion of cells in the S phase and the presence of numerous cells that failed to demonstrate thymidine uptake. These disturbances in the erythroblastic cell cycle would be in keeping with ineffective erythropoiesis (Abdalla,1990). This is usually echoed in the peripheral blood by the lack of a reticulocytosis which is appropriate for the degree of anaemia. The mean reticulocyte count was ~ 4% (ranging from 0.5 - 18.2%) and the mean haemoglobin was 11.2 g/dl, possibly indicating that in this study ineffective erythropoiesis was not the main contributor of anaemia. We must bear in mind however that the reticulocyte counts were not documented in the majority of the study patients, and that the contribution of ineffective erythropoiesis to the anaemia may be underestimated.

A group of researchers from Denmark studying the effect of bone marrow inhibition in the pathogenesis of malarial anaemia concluded that malarial infection is associated with

inhibition of erythropoiesis (Kuitzhals et al,1997). As only two marrows were available for assessment in this study group, this theory was difficult to confirm.

In the endemic areas the contribution of malaria to the development of anaemia, especially in children, is often difficult to determine as there are often other causes of anaemia e.g iron deficiency and haemoglobinopathies (Newton et al,1997). Of note was the fact that within the paediatric group , a more microcytic type of anaemia was evident. Ideally iron studies should be done to exclude the presence of iron deficiency. Morphologically, however, there were no gross features to suggest this or the presence of a haemoglobinopathy.

5.5.2.1.1 Blood transfusion practise in the malaria patients

Sixteen percent of the study patients received a blood transfusion with anaemia as the main indication. Generally a transfusion will be given if there is a severe anaemia (HB <5 g/dl), or if the patient is symptomatic or is haemodynamically unstable. For the purpose of the study the transfusion data was documented based on whether a severe anaemia was present or not. Among this group of patients one case fatality was recorded, in a pregnant female who received a multiple transfusion for a HB of 7 g/dl. With greater improvement in blood screening especially for viral infections like HIV,and the hepatitis viruses, blood transfusion has become an increasingly safer practise for the management of anaemia. Nevertheless caution should be exercised not to unnecessarily transfuse patients with excessive amounts of blood products. Four of the forty-two patients received more than 2 units of blood. Five out of forty-two patients received an exchange blood transfusion, the main indication being hyperparasitaemia. The aim of treatment with exchange blood transfusion is to reduce the high level of parasitaemia as quickly as possible, often in conjunction with intravenous quinine. The rationale is that

since quinine is relatively slow acting, the exchange transfusion will reduce the parasitaemia initially sufficiently until the effect of quinine is optimal. It also has the advantage of removing any soluble toxins and pathogenic mediators (Warrell et al, 1990; Eisenman et al, 1995). This modality of treatment however is still controversial (Marik, 1989; Miller et al, 1989; ed. Lancet 1990; Fontes and Munhoz, 1996).

Another interesting finding, which to my knowledge has been well documented in the literature as an important indicator of severe malaria, is the WCC. A univariate analysis of the continuous data identified a significant difference in the leucocyte counts between the survivors and those that died. The WCC was found to be significantly higher in those that died ($p=0.0281$).

5.5.2.2 Analysis of chemistry data

In non-immune adults, up to 1/3 of the individuals with falciparum malaria may have biochemical evidence of renal dysfunction (Francis and Warrell,1993). This may be related to the underlying infection with obstruction of glomerular capillaries by PRBC, or secondary to haemolysis, but may also only be related to simple dehydration and renal function is then simply restored by rehydrating the patient with intravenous or oral fluids. Previous studies have identified renal dysfunction and especially renal failure in high numbers ; Soni and Gouws (1996) had up to 17% of their patients presenting with renal failure. In a retrospective study of malaria presenting at the Johannesburg Hospital, Jairam et al (1990) documented 9% of cases with acute renal failure. Analysis of the data in this study revealed only 3% of patients with biochemical evidence of renal failure (creatinine > 265μmol/l). Most authors identify high urea levels to be significant predictors of poor outcome. Multivariate analysis and logistic regression models found

high creatinine levels to be significantly associated with reduced survival, p=0.00852 and p=0.0016 respectively. The mean serum creatinine level was 238.3 μmol/l in those who died as compared with 104.9 μmol/l in the survivors. Surprisingly no statistical difference (p=0.2032) was demonstrated between the means. A possible explanation for this includes the wide standard deviations derived as well as the small numbers for this variable, in the non-survivors. In further contrast to what has been reported in the literature by the group in Natal who documented hyperbilirubinaemia and jaundice in 10.4% of their cases (Soni and Gouws,1996), 32% of the study patients had evidence of some liver dysfunction as noted by an elevation of the liver enzymes and raised bilirubin levels. Impairment of liver function is associated with severe malaria. It is often difficult to ascertain precisely what degree of dysfunction is present, as measurements of bilirubins and liver enzymes may be elevated in the presence of co-existing haemolysis. More accurate measurements of hepatic dysfunction include the assessment of coagulation abnormalities resulting from failure to produce clotting factors, hypoalbuminaemia and reduced clearance of alanine and lactate (Warrell et al,1990). Of the parameters described above only albumin levels were determined. The mean albumin level was 35.7 g/l which is well within the normal range. This suggests that the results of the liver function tests were probably influenced by underlying haemolysis rather than by true hepatic failure. An accurate assessment however needs to take all the above factors into account.

Hypoglycaemia is being recognised as an important manifestation of falciparum malaria. In the present study 5% of the patients presented with low glucose levels (glucose < 2.2 mmol/l). Hypoglycaemia was significantly associated with the pregnant state. The cause of the low glucose levels in these

patients is difficult to determine. It may be associated with increased glucose consumption by the host (fever and infection) and/or by the parasite (anaerobic glycolysis). Impaired gluconeogenesis or glycogen depletion has also been implicated; this may be the result of high circulating TNF levels and the presence of increased plasma concentrations of insulin. Hypoglycaemia is also an important complication of treatment with certain antimalarial drugs like the cinchona alkaloids, quinine and quinidine. In vitro quinine has been demonstrated to stimulate the secretion of insulin from the islet cells. This usually results in decreased hepatic gluconeogenesis and increased uptake of glucose by the peripheral tissues (Warrell et al, 1990).

5.5.2.3 Serological data and the assessment of immunity

One of the aspects that this project tried to assess, was the issue of underlying immunity. In order to determine this the IFAT, looking for the presence of malaria antibodies, was used together with a quantitation of IgG and IgM levels, at the time of presentation. The results were compared between a group of patients with documented previous exposure to malaria (24 patients;~12%) and a 'non-exposed' group ,which comprised the majority of the study cases tested. Most of the patients had a rise in the total immunoglobulin levels, which is a common observation after an acute malarial attack. IgG levels ranged between 7.9 - 44.5 g/l and IgM levels between 0.6 - 5.1 g/l. The normal ranges in adults are 10-20 g/l and 0.5-2.7g/l respectively. What proportion was specific antimalarial antibody was not determined, as immunoglobulin subtypes were not differentiated.

Overall the IFAT was difficult to analyse accurately, and it was difficult to correlate the IgG and IgM titres against the IgG and IgM levels for the reasons mentioned above. Interpretation of the IFAT was based on recommendations by the manufacturers of the

kit. The test appears to be highly sensitive with 94% of the patients having positive titres (IgG ≥1:80), however in 3% of cases the test was negative (IgG <1:20). These patients had thin smears positive for falciparum malaria. The reason why these individuals would have a negative test is uncertain. The test appears to be highly subjective, and even a skilled microscopist may experience some difficulty when examining the samples under the fluorescent microscope. It is possible that these few cases may have been weakly positive due to the presence of an early infection.

In the ideal situation where individuals have had no previous exposure to a particular antigen, the initial antibody 'primary' response is predominantly IgM (Marsh, 1990). Subsequent re-exposure results in a secondary response and the predominant immunoglobulin then is of the IgG type. One would therefore expect to have the majority of the patients in this study having a strong IgM response, however only 65% had highly positive titres, this included 10/24 patients who had a history of previous exposure.

Those individuals who had borderline or negative titres ie. <1:20; 1:20;1:40 may have had a non-falciparum infection. This, however, would only account for 6% of the patients. Falciparum antigen has been known to cross-react with antibodies directed against other species, although at a lower titre. This can be overcome by ensuring that the antigen that is used is adequately purified (Campbell et al, 1987). Another hypothesis which may explain the low incidence of positive IgM titres is the possibility of the presence of a different strain of *Plasmodium falciparum* against which antibodies have been made. These antibodies may therefore not react with the antigens used in the IFAT.

Among the individuals with a history of previous infection only 6/24 (25%) had low IgM titres, the remainder as described above showed an IFAT which would be in keeping with recent active infection. The explanation as to why these few patients have higher IgM titres includes the possibility that they may have lost a previous 'immune' state, and have become susceptible to acute infection again. Alternatively the IgM titres may be reflecting a false positive result (Dr John Frean - pers.comm.)

The only consistent finding among the majority of the previously exposed group (17/24) was the presence of a low level parasitaemia (< 1% to 1%), which may be indicative of a low grade chronic infection .This however did not correlate well with the IFA test. Perhaps a better way to assess underlying immunity is by measuring specific malaria induced subclasses of immunoglobulin e.g IgG1 and IgG3.

5.5.2.4 Analysis of pregnancy data

The study patients were divided into two main categories: - the previously exposed 'immune' patients, and the non-immune cases. Within the latter an additional two groups were identified, that of the pregnant women and those patients who required ICU admission. The first two categories have already been discussed in some detail.

Analysis of the pregnancy data revealed what already has been documented in the literature, that pregnancy is associated with an increased susceptibility to malaria infection and that there is also an increased risk of foetal loss. Fievet et al (1995) investigated the mechanisms underlying the increased susceptibility to malaria in pregnant women, by studying a group of Cameroonian primigravid . Their findings suggested that an immunosuppression process occurs during pregnancy and this immunosuppression appears to be due to a defect in the antigen presentation and processing mechanisms, with an altered capacity of lymphocytes to proliferate, and a

reduced IL-2 production. Similar defects,however were also present in response to non-malarial antigens. The authors postulate that the alteration of malaria immunity might be a non-specific phenomenon, but in pregnancy and particularly in the primigravidae,this alteration of the immune response to malarial antigens is possibly restricted to a particular subset of lymphocytes.

5.5.2.5 ICU admissions

Review of the ICU data revealed a few indicators that may be used in the future to identify which patients are candidates for early intensive care, to reduce morbidity and mortality from malaria infection. Fifteen patients were admitted into ICU, of which five died. The important parameters that should be assessed on presentation of the patient with malaria, and then on subsequent follow up of the patient in the general ward include: the presence of a hyperparasitaemia (especially with falciparum malaria), the presence or development of renal failure, cerebral malaria, hypoglycaemia and the pregnancy state of the individual. Finally if any of the above parameters are associated with severe anaemia and/or a high WCC the patient should be considered for ICU care. Unfortunately this is often easier said than done as entrance into the ICU is often limited due to the availability of beds. When the situation arises that the patient cannot have immediate access to intensive care in an ICU facility, if the clinicians are aware of the high risk patient they may be able to provide more aggressive therapy earlier, i.e exchange transfusions and renal dialysis in addition to the routine chemotherapy.

5.5.2.6 Treatment

At the Chris Hani/Baragwanath Hospital the mainstay of treatment for acute infection is oral quinine, given in 88% of cases, alone or in combination with drugs like tetracycline

and doxycycline. Quinine may initially be given intravenously, as was observed in 12% of the study cases, in the event of a severely ill patient and/or patients who are unable for one or other reason to take the drug orally. Quinine and tetracycline/doxycycline therapy was initiated simultaneously. After seven days quinine was discontinued and the course of tetracycline/doxycycline was completed after fourteen days. In the event that the patient was discharged prior to finishing the prescribed treatment, the balance of the medication was given to take home. The only other treatment option available in the event of failure with quinine, is Fansidar (although this was not used in any of the patients during the course of the study).

With the emergence of resistant strains, why is it that we can still rely on quinine, after centuries of use ? Meshnick (1997) gives three possible explanations as to why quinine is still effective as an antimalarial drug:

a) One of the mechanisms of resistance is the emergence of new parasite strains (mutations) that are no longer sensitive to a particular drug; it is postulated that the intraparasitic target for quinine is so specific that mutations of this target site are occurring at a very slow rate,

b) there may be some parasite strains that have developed resistance to quinine, relative to those strains which existed centuries ago, and since this resistance may have occurred gradually we may not have noticed that the requirements (i.e doses) of quinine have increased, and

c) the use of quinine may not have been so intense that it exerted evolutionary selective pressure until now.

Treatment for malaria can be divided into (i) therapy for uncomplicated disease, (ii) management of severe /complicated malaria, and within these two groups the special considerations that should be given when treating children and pregnant women. The recommended drugs and treatment regimens have been reviewed in detail and will not be discussed further (Kozarsky and Lobel, 1994; Olliaro and Trigg, 1995; Schultz et al, 1995; Department of Health, 1996; White, 1996).

Among the treatment options is the entity of self administered therapy for acute malaria. This is often employed in areas where the distance and the cost of seeking care from health services may be great, or when traditional care from a healer is considered more appropriate. The major shortfall with this is the lack of public awareness on malaria and the subsequent lack of appropriate use of antimalarial drugs; this has often been associated with further development of drug resistance (Foster, 1995; Ruebush et al, 1995; Mnyika et al, 1995; Schlagenhauf et al, 1995). Perhaps one of the ways to overcome this is to develop an easy to use and simple to interpret test, in a kit form that will be suitable for self-diagnosis. Travellers can then start appropriate therapy once a positive test is encountered, until medical advice is able to be sought (Schlagenhauf et al, 1995).

Malaria continues to be one of the most important health problems in the World but especially in sub-Saharan Africa. In South Africa, only a few areas are endemic to this infection, the remainder of the problem stems from imported malaria.

Despite efforts to control this disease worldwide and locally, the incidence appears to be rising. Whether this is due to emerging resistant strains to antimalarial chemoprophylactics, or aggrevated by climactic changes like global warming and the El Ninõ effect, or whether it arises from basic ignorance of the disease among the affected populations and among travelers, is difficult to ascertain.

At present the incidence of malaria appears to draw strength from all of the above.

6.0 CONCLUSION

The important aspects that were revealed in this dissertation have been summarised below:

a) The incidence of malaria at the Chris Hani/Baragwanath Hospital is increasing annually with a significant rise in the number of admissions during 1996 compared with those noted in 1994 and 1995.

b) There is a distinct seasonal pattern which appears to coincide with holidays, viz Christmas and Easter.

c) The majority of cases are due to imported malaria from the neighbouring countries. Most (58%) of the imported malaria infections (predominantly *Plasmodium falciparum*) were acquired in Mozambique. Four patients, who presented with falciparum malaria, had no travel history and the source of infection is unidentified in them. In one of the cases the possibility of 'taxi rank' malaria exists.

d) The most common infecting species was *Plasmodium falciparum* (95.1% of cases).

e) Climactic data did not support the possibility of transmission of infection in Soweto. There is also no definitive proof that the malaria transmitting vectors (*Anopheles gambiae* and *Anopheles arabiensis*) are found normally in Gauteng. When asked whether it would be possible for 'imported' mosquitoes to breed with local species,

colleagues from the Medical Entomology department of Tropical Diseases of the School of Pathology, at the South African Institute for Medical Research, responded that it would be very unlikely for breeding colonies to be established with local *Anopheles* mosquitoes (Drs M. Coetzee and R. Hunt - pers. comm.)

f) Clinical and laboratory results confirmed findings documented in the literature by other authors. The prevalence of complicated disease among this study group seemed less than previously reported in other South African studies.
Hyperparasitaemia and renal failure (based mainly on the creatinine level) were found to be significant predictors of poor survival. In addition to the morbidity and mortality associated with malarial anaemia, the WCC (and particularly a raised count) was found to be an important prognostic indicator.

g) The findings among the pregnancy group confirmed the poor outcome associated with this infection, both for the mother and the foetus.

h) The main criteria for ICU admission (provided availability of beds) appear to include hyperparsitaemia, the presence of renal failure, cerebral oedema and the pregnancy state. Additional factors that need to be taken into consideration are hypoglycaemia, and the presence of a severe anaemia and a raised WCC.

i) The mortality rate was 3% and the deaths were mainly associated with the adult group. Only one of the six patients who demised was a child. This is in contrast with the high childhood mortality noted in endemic areas.

j) The underlying immune status of the patients was difficult to assess accurately by using the IFAT. In the patients with a history of previous exposure the results of the test were not consistent. Among the patients with no previous exposure to malaria, the test successfully identified acute and active infection. The IFA test was reported as negative in three patients, who had documented disease on thin and thick smears. The reason for this is uncertain, however the possibility of an early infection cannot be excluded. Factors that need to be taken into account when interpreting this test is the presence of non-falciparum infections that may cross-react with the falciparum antigens, and in 'immune' individuals the loss of a previously acquired immunity, with the presence now of a 'primary' response to an acute infection. Immunity to malaria is reported to be acquired slowly but may be lost rapidly if continuous exposure to the malarial antigen is lost.

k) The single most important fact that became apparent was the lack of knowledge regarding malaria and its consequences, even among individuals who regularly visited endemic areas. In those individuals who sought pre-travel advice, the use of prophylactic drugs was inconsistent and was not accurately completed. In addition the drugs prescribed were often not suitable for prophylaxis.

l) Within the hospital setting quinine is still the mainstay of treatment for acute infections. Specialised therapy like exchange blood transfusions for hyperparasitaemia were limited but when used, successfully reduced the parasitaemia. Out of five patients who received this modality of therapy in conjunction with quinine, one individual died of other complications.

It is apparent that the repertoire of drugs for malarial prophylaxis and treatment is limited at the Chris Hani/Baragwanath Hospital. The reason for this is uncertain but may be due to the cost of these drugs. However with the emergence of CQR and now also quinine resistance, the range of drugs needs to be increased, if effective therapy is to be given for malaria patients. With the emphasis now on prevention and primary health care, the role of providing malarial prophylaxis within the hospital sector will need to be expanded.

7.0 RECOMMENDATIONS

In 1957 the World Health Organisation launched its first formal global campaign for the control of malaria. Now, more than forty years later, the WHO are still fighting an apparently losing battle. The latest recommendation is that malaria control be an integral part of the national primary health care system. With this is mind, the MANTEAU project was launched in 1993: Managing Tropical disease Through Education and Understanding; the ultimate aim being to provide a well trained workforce who are motivated and knowledgeable in the implementation of new technology to supplement traditional methods of education and management.

The goals of this programme include:

a) human resources development through on-site training and action learning.

b) capacity building at local and national levels through enhancing comunications and infrastructures.

c) strengthening national programmes in developing countries through the development of tools and techniques for improved management and control involving training, data management and decision support.

d) sustained tropical diseases management ensuring quality control through improved clinician support.

What can we do on our part to assist such a programme ?

In my opinion one of the most important 'needs' in order to make a programme like this successful is the development of health information systems. These may be hospital or clinic based.

The first assignment of such an 'information system' would be to make a strong statement about the extent of the malaria problem. This can take the form of a 'malaria awareness day', where members of the public and in this case individuals who commute to and from the Chris Hani/Baragwanath Hospital and the surrounding clinics, can be bombarded with information regarding the transmission of malaria and how best to guard against this infection. Posters and pamphlets are an easy and cost effective way of achieving this. The most appropriate time of the year to launch this campaign would be in December before the Christmas holidays. The use of the media, mainly newspapers and radio, and to a lesser extent the television (as some of the population that we wish to target may not have such a luxury) may be useful to promote this event, and later may even be utilized to spread malaria awareness through special (malaria) education broadcasts/editions.

The next step would be to target the health care providers, and to regularly update these individuals with the latest developments in prophylaxis and treatment. This in part is already in motion. With the collaboration of the Department of Health and the TPS Drug Information Centre, guidelines for the prophylaxis and the treatment of malaria are being circulated in journals to which clinicians have access.

In our multicultural societies, health care providers include traditional healers, herbalists, homeopaths and the hawker who sells his wares on the street corner. We therefore need to educate these individuals as well, and possibly to recruit their assistance in the fight against malaria.

As part of the MANTEAU project the WHO released a booklet for the 'non-pharmacist' selling antimalarial drugs. This originally was aimed at market place shops in malaria endemic areas, however it can very easily be applied to our local situation. The booklet provides comprehensive information on malaria, who gets it and how it is acquired. It has a section which deals with the available drugs for the prophylaxis and treatment of malaria, with dosage regimens illustrated in table forms. It gives advice on what to do in the event the treatment option does not improve the patient's condition and also describes the features of severe malaria and gives recommendations as to the best time to seek medical advice. As most of our malaria is imported, and as a result of travel to an endemic area, we may be able to provide travel information in the form of this booklet.

In addition one could include a simple to use diagnostic test, like the antigen capture dipstick tests that are available, for self diagnosis in the event of symptoms. Combine this with (i) adequate prophylactic cover in the form of chloroquine and proguanil, (ii) a topical or aerosol insecticide, and (iii) standby treatment in the event of an acute infection e.g quinine, and you have the 'new improved malaria travel kit'(Schlagenhauf et, 1995). However, for such a venture to be successful there will be the need for adequate education of those individuals who acquire the kit, in order to avoid confusion

regarding the treatment protocols included. The kit can be made available from state hospitals, clinics, over the counter in privately owned pharmacies, even at border posts. It should ideally be manufactured by a pharmaceutical company and made available to the Department of Health, for distribution to the relevant sites as mentioned above. The initial costs may be high (alternatively it may be subsidised by the manufacturing company) but until a better option is available i.e the long awaited 'holy grail' - the antimalarial vaccine, our choices are limited.

In the interim, a more realistic solution would include the availability of more prophylactic agents at health clinics and state hospitals, an issue which the Department of Health should list as number one on their agenda.

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Malaria is a common and serious disease. Each year hundreds of travellers are at risk of acquiring this infection, and many more do fall ill with malaria, and even die. The biggest problem is the lack of knowledge about this disease and what causes it. With prevention and treatment of malaria becoming increasingly more difficult, we need to educate our populations to be more aware of the dangers associated with malaria, to take adequate precautions, and above all else to seek urgent medical advice in the event of suspected infection. Malaria especially falciparum malaria although deadly is very amenable to therapy and can be limited if it is detected and treated early.

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APPENDIX A

SUBJECT INFORMATION AND CONSENT FORM

You have been diagnosed as having Malaria. This condition results from the bite of a certain type of nosquito, which is infected with the organism (parasite) which causes the infection. Individuals that travel to areas where these mosquitoes are plentiful, without adequate medical protection usually become ill after they have become exposed to mosquito bites. The course of the disease is usually benign, however some individuals may develop serious and often life threatening complications e.g. renal failure and central nervous system involvement (cerebral malaria). Early diagnosis and treatment leads to a speedy and uneventful recovery. In January - March 1994 about 100 patients were admitted to Baragwanath with the same condition that you have. This reflected a very high number of affected individuals.

I am conducting a project on malaria, to try and answer some important questions which may provide some insight to the high incidence of this infection in Soweto.

If you agree to take part in my study you will be expected to answer a very simple questionnaire, and allow me to do a clinical examination not unlike the one your doctors have done on your admission. This is done confidentially. You might also be expected to undergo one or two extra blood laboratory investigations which will provide us with important information with regards your condition. Your refusal to participate in this research project will not affect your medical care in any way.

At the bottom of this information sheet you will find a consent form which you are expected to sign if you agree to be entered in my study. If you are under 18 years of age, a guardian or next of kin will be expected to sign on your behalf.

*DATE.*_____ *DOCTOR.*_____

*I*_____ *fully understand the information that was given to me by*_____ *and agree to take part in the abovementioned study. I also understand that I may withdraw my consent and discontinue my participation at any time, and that this will not affect my regular treatments at this hospital.*

*DATE.*_____ *PATIENT.*_____

GARDIAN/NEXT OF KIN: _____

WITNESS: _____

APPENDIX B

RETROSPECTIVE STUDY
PATIENT INFORMATION SHEET

SOCIAL HISTORY

Name: **Age:** **Hosp.No:**

Gender: **Date of admission:**

Present address: **Permanent address:**

Nationality: **Income:**

Travel history: **Prophylaxis:**

MEDICAL HISTORY

Signs and Symptoms:

Gestation:

Treatment: **TTO:**

OUTCOME:

Discharged **Demised**

APPENDIX C

QUESTIONNAIRE
MALARIA

Name: _____ *Hospital No:* _____

Age: _____ *Sex:* _____ *Occupatiion:* _____

Address: _____

Tel: No.: Home: _____ *Work:* _____ *Ward:* _____

Date: Admitted: _____ *Referred:* _____

Nationality: _____ *Income:* _____

HISTORY:

_____

_____

_____

_____

GENERAL:

Anorexia: _____

Fever: _____

Night Sweats: _____

Pruritis: _____

General well being (malaise): _____

Fatique: _____

Weakness: _____

Lymphadenopathy: _____

Joint pains: _____

Thrombosis: (DVT, stroke) _____

Headaches + visual disturbances: _____

Amenorrhoea: _____

Impotence: _____

Bleeding: (site, duration, amount) _____

Epistaxis: _____

Haematysis: _____

Haematemesis + Malaena: _____

Haematuria: _____

Menorrhaagia: _____

Mucous membranes + Gums: _____

Skin (purpura): _____

Venepuncture sites: _____

Haemarthrosis: _____

SYSTEMIC ENQUIRY:

Head & Neck: _____

_____

Respiratory: _____

_____

Cardiovascular: _____

Gynae: Gestation: _____

DRUG HISTORY:

MALARIAL PROPHYLAXIS: Was it given/Advised ? _____

What Drugs were Issued ? _____

Who prescribed the Drugs ? _____

For how long were they taken ? _____

OTHER: _____

HOSPITAL THERAPY: _____

PAST HISTORY: *(Malaria/TB/HPT/Diabetes/Malignancy/Chemotherepy/Radiotherapy/Illness/Operations)*

_____

TRAVEL HISTORY: *Destination ?* _____

Duration of Visit ? _____

Frequency of Travel to Malaria Areas ? _____

CLINICAL EXAMINATION:

T° _____ PR: _____ BP: _____

Colour: _____ Pallor: _____ Cyanosis: _____

Jaundice: _____ Oedema: _____

Lymphadenopathy: _____

Head & Neck: _____

CVS: _____ Chest: _____

CNS: Fundi: _____

Sensory: _____

Motor: _____

Cranial Nerves: _____

Cerebellar: _____

Reflexes: _____

FINAL DIAGNOSIS:

_____

OUTCOME OF DISEASE:

_____

LABORATORY RESULTS:

APPENDIX D

STANDARD OPERATING PROCEDURE FOR A DIFFERENTIAL COUNT
Proc#HCEN0038.MET
(SAIMR *Department of Haematology*)

CLINICAL ASPECTS:

1.1 Physiological aspects,clinical significance and indications for the test

A differential is not only requested for the diagnosis of haematological disorders but also to provide information for diagnosis of non-haematological diseases, for indicating side effects of chemotherapy, and for monitoring patient therapy. Reasons such as these make it essential that a blood smear be prepared correctly and examined in such a way as to provide the physician with an accurate interpretation. Blood smears are prepared from EDTA anticoagulated blood to minimize degenerative changes in the blood cells. The collection tube must be completely filled with the appropriate amounts of blood so that it can mix with the anticoagulant. If there is an excess of anticoagulant, artefacts will occur. To ensure good preservation of cellular morphology, blood smears should be made as soon as possible and no later than 3 hours after collection.

SLIDE PREPARATION
PRINCIPLE:

A small drop of blood is placed near the frosted end of a clean glass slide. A second slide is used as a spreader. The blood is streaked in a thin film over the slide. The slide is allowed to air dry and is then stained.

SPECIMEN:
EDTA, Finger prick

Patient preparation:
Nil

Type:
EDTA blood or unanticoagulated capillary blood.

Handling conditions:
The EDTA blood must not be older than 3 hours.

EQUIPMENT AND MATERIALS:

Equipment:

1. Glass slides 3x1 inch (precleaned with frosted ends)
2. Glass spreader (width slightly less than 3")
3. Capillary tubes, plain.

PROCEDURE:

1. Clean slides are essential.
2. Make a spreader with a perfectly smooth edge by breaking o.5cm from the width.
3. Fill a capillary tube three-quarters full with the EDTA blood.
4. Place a drop of blood about 2 mm in diameter approximately 10 mm from the frosted area of the slide.
5. Place the slide on a flat surface, and hold the narrow side of a the non-frosted edge between your thumb and forefinger.
6. With your right hand, place the smooth clean edge of the narrower side of the spreader on specimen slide, just in front of the blood drop.
7. Hold the spreader slide at a 30 degree angle, and draw it back against the drop of blood.
8. Allow the blood to run along the width of the spreader by capillary attraction.
9. Push the spreader forward with one light,smooth, and fluid motion. The weight of the slide should be the only pressure applied. A thin film of blood in the shape of a bullet with a feathered edge will remain on the slide.
10. Allow the blood to dry completely before staining. This can be accomplished by waving the slide in the air vigorously or by placing the slide in a stream of cool air.

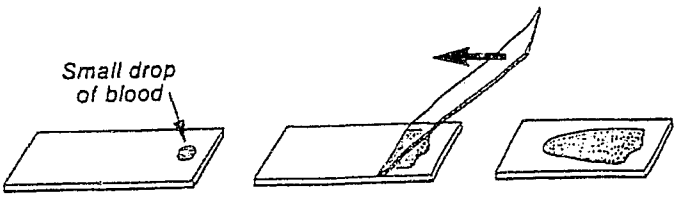


Figure A: Preparation of a peripheral blood smear, slide to slide technique

COMMENTS AND LIMITATIONS OF THE PROCEDURE:

1. A good blood film preparation will be thick at the drop end and thin at the opposite end.

2. The blood smear should occupy the central portion of the slide and should not touch the edges nor extend into the last 15 mm of the slide.
3. The thickness of the film is determined by:
   -the volume of blood
   -the angle of the spreader
   -the speed of spreading
   Thicker films can be produced by increasing the angle of the spreader and the speed of spreading, and vica versa to produce thin films.
4. The wedge slide is one of the most popular methods for producing a blood film, but it does not produce quality smears. The WBC's are unevenly distributed and RBC distortion is seen at the edges. Smaller WBC's such as lymphocytes tend to reside in the middle of the feathered edge. Large cells such as monocytes, immature cells and abnormal cells can be found in the outer limits of this area.
5. Excessively thin films will distort red cell morphology, with a tendency to accumulate WBC's at the margin and the tail.
6. Thick films will have insufficient area where the red cells are spread without overlapping and the WBC morphology will be obscured.
7. Do not tap the capillary tube on the slide to try to get more blood out of the capillary tube. This causes the RBC's and the WBC's to disintegrate.

STAINING OF THE BLOOD FILM

See staining section of the Manual for manual and automated staining techniques.

EXAMINATION OF THE PERIPHERAL BLOOD SMEAR

There are several necessary steps in the examination of a peripheral blood smear:

LOW POWER (X10)SCAN

1. Determine the overall staining quality of the blood smear.
2. Determine if there is a good distribution of cells on the smear.

a. Scan the edged and centre of the slide to be sure that there are no clumps of RBC's,WBC's or platelets.

b. Scan the edges for abnormal cells.

3. Determine the WBC estimate.
4. Find an optimal area for the detailed examination and enumeration of cells.

a. The RBC's should not quite touch each other.

b. There should be no areas containing large amounts of broken cells or precipitated stain.

c. The RBC's should have a graduated central pallor.

THE 50X OIL IMMERSION EXAMINATION

Note: the 50x oil immersion objective is recommended for the performance of the 100-cell differential for the experienced morphologist because it enhances speed without sacrificing accuracy especially on low WBC counts (below 4.0 x 10^9/l. It is

recommended that 100 x oil immersion objective be used by beginner morphologicts until they become familiar with the various cell types.

1. Various counting patterns have been suggested to overcome the tendency, present even in the best made wedge films, for the neutrophils to accumulate at the edges and in the tail of the film. In an attempt to represent fairly the neutrophils at the edge of the film the "battlement' pattern includes the edges of the film, which, if the slide is poorly made, will result in counting excess neutrophils.

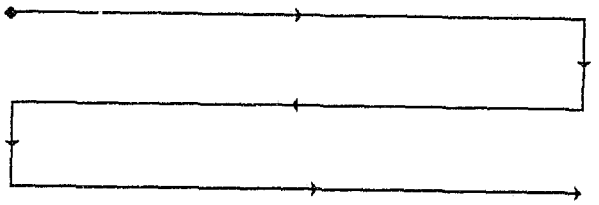


Figure B: Scanning pattern illustration

Counting all the leucocytes in a longitudinal strip from head to tail of the film has been recommended on the premise that in a well-made film each strip will represent the blood drawn out from a small volume of blood when it has spread out between the slide and the spreader. The disadvantage of this method is that in the thicker part of the film WBC's appear smaller and condensed making identification more difficult.

2. Perform a 100 WBC differential count and report the differential as a percentage and as an absolute number.
3. Correct any total WBC counts that has greater than 10 nucleated red blood cells (NRBC) per 100 WBC's.

i When performing the differential, do not include NRBC's in your count, but report them as the number of NRBC's/100 WBC's.

ii Use the formula under the calculations heading to correct the WBC counts.

4. Perform a WBC estimate and evaluate the morphology (nuclear and cytoplasmic).

i Using 50x objective count the number of WBC's in 10 fields. These must be counted in an area where the RBC's *slightly* overlap.

ii Divide by 10.

iii Multiply by 3.0 to get the estimate count.

5. Evaluate the red cells for size,shape,colour,and inclusion bodies. This must be done in an area where the red cells are evenly dispersed and are just separating. At least 10 fields should be scanned. In thick areas RBC morphology cannot be

seen and in the thin areas the RBC's tend to look hyperchromic, and variation in shape is lost.

6. Perform a platelt estimate and evaluate platelet morphology.

i Using 100x objective count the number of platelets in 10 fields. These must be counted in an area where the red cells are evenly dispersed.

ii Divide by 10.

iii Multiply by 20.

CALCULATIONS:

$$\text{Absolute value } (\times 10^9/l) = \frac{\% \times \text{WBC count}}{100}$$

$$\text{Corrected WBC } (\times 10^9/l) = \frac{\text{WBC} \times 100}{(\text{NRBC} + 100)}$$

PROCEDURE NOTES:

Reference Ranges: please see MATERIAL AND METHODS, pg 77-78

Procedure for Abnormal Results:
As per laboratory SOP

Reporting format:
APEX or equivalent

LIMITATIONS OF THE PROCEDURE:

1. The best films are made from unanticoagulated blood. If an anticoagulant is used the film should be made immediately if possible, or nomore than 3 hours after collection.
2. Comments made in the section covering slide preparation are also applicable here.
3. Consultation should be sought for abnormal smears and for all new cases with abnormal haematological results.
4. Automated differential machines each have their limitations with regard to their differential counts. The technologists must be familiar with the laboratory's instruments.

REFERENCES:

1. Peripheral blood cell morphology. 1983
2. Hall R. and Malia R.G. Medical Laboratory Haematology, 2nd Edition

APPENDIX E

STANDARD OPERATING PROCEDURE
MALARIA INDIRECT FLUORESCENT ANTIBODY TEST
(SAIMR *Department of Parasitology*)

PRINCIPLE:
Antibodies in patient's serum bind to *P.falciparum* on a slide and are detected by antihuman antibodies labelled with FITC. A fluorescence-equipped microscope is used to read the test.

EQUIPMENT AND MATERIALS:
Fluorescence(FITC)-equipped microscope
Magnetic stirrer
Antigen slides
Phosphate buffer (PB) pH 7.4
Polyvalent FITC-labelled antihuman globulin *
Evan's Blue diluent(1:100 in PB)
Positive and negative serum specimens
Pipette with tips

PROCEDURE -STEPWISE:
Dilutions: to make 1/20 dilution of test serum, positive control and negative control sera, place 500 µl of phosphate buffer in an Eppendorf or similar tube. Take out 25µl and discard. Add 25µl of test serum,control serum or negative serum to the buffer and mix; for the test serum make doubling dilution up to 1/160 dilution.
Antigen slides: Remove the slides with antigen from the freezer and place in a Coplin jar with phosphate buffer and magnetic stirrer. Wash for 10 minutes. Wipe the slides with tissue paper except the area with antigen.
Test:

1. Place 20µl of the dilutions of test serum,positive serum and negative serum in wells on the slide.
2. Incubate at 37 degrees for 30 minutes in a humidified chamber.
3. Place the slide(s) into a Coplin jar with phosphate buffer, a magnetic stirrer and with 3 changes of buffer for 10 minutes each.
4. Wipe excess buffer off the slide(s) with tissue paper but do not touch well contents.
5. Place 20 µl of polyvalent conjugate * at the appropriate dilution in Evan's blue/buffer (dilution factor varies with each batch; consult package insert) onto each spot and incubate at 37 degrees for 30 minutes ina humidified chamber.

6. Repeat step 3
7. Place coverslip over the spots and examine with fluorescent light under x 100 and x 400 magnification.

REPORTING RESULTS:

Under ultraviolet light the plasmodia (rings and schizonts), which have reacted with positive sera are seen as bright yellowish-green against a reddish-brown background. Negative specimens show merely as reddish-brown.

* For the purposes of this study, the test sera were treated directly with specific IgG and IgM (monovalent conjugates) antihuman globulins.

APPENDIX F

STANDARD OPERATING PROCEDURE FOR THE DETERMINATION OF IMMUNOGLOBULIN LEVELS - e.g IgG (SAIMR Department of Chemical Pathology)

CLINICAL DETAILS:

1.1 Physiological Aspects:

IgG is the predominant serum immunoglobulin comprising about 75% of total serum protein. There are 4 sub classess of IgG in normal serum.IgG is of particular importance in the bodies long term defence against infection. IgG deficiency is associated with recurrent and occasionally severe pyogenic infections. IgG is the only immunoglobulin to cross the placenta. IgG synthesis and serum levels are increased in response to chronic or recurrent infections or auto-immune diseases.

1.2 Clinical Significance:

Auto antibodies are of the IgG class e.g antinuclear antibody, and anti-basement membrane antibodies. A significant increase in IgG also occurs in patients with multiple myeloma of the IgG type; this is the most common form of multiple myeloma.

1.3 Indications for the test:

PRINCIPLE:

As the central component of the Array Protea System, the rate nephelometermeasures the intensity of the light as it is scatteres by particles in suspension in a semi-disposable flow cell when a beam of light is passed through the cell. The particles are formed by the immunoprecipitation reaction that occurs when a specific antibody is brought into contact with the specific antigen. The resulting formation of complexes and the consequent change in the intensity of scattered light occurs at a rate that increases gradually at first, then rapidly, and finally proceeds through a peak rate of change (peak rate value) for the cmponent being analyzed. For Beckman specific protein test kits, the analyzer electronics system derives the peak value for the rate-of-change from the scattered light signal, converts that value into concentration units, presents the results on a CRT, and transmits it to the printer.

ANTIGEN EXCESS DETECTION:

For a constant amount of antibody, the magnitude of the rate signal will increase as antigen levels in the serum sample increase until excessive levels of antige cause a diminished rate response. It is therefore possible for two significantly different concentrations of antigen in the serum to yield the same rate response. The Array Protien System is programmed with a standard antigen excess check mode to discriminate between a reaction that is in antigen excess and one that is

in antibody excess. This mode tests for the presence or adsence of unbound (excess) antibodies in solution after the primary reaction, in reactions identified by the program as being ambiguous (i.e when the rate units measured could be either antigen excess or an antibody excess primary reaction)

OUT-OF-RANGE DETECTION:
The concentration of each lot of antibody in a Beckman Specific Protein Test Pack has been adjusted to a level compatible with the prescribed dilution of serum samples so that the measuring range of the reagent will cover approximately 90% of expected values. Each antibody card contains information which defines out-of-range high and out-of-range low limits for each assay. samples producing rate values above or below these limits will automatically be retested at the next appropriate dilution, unless a non-standard dilution has been selected for the chemistry which is below the limit set on the card.

SPECIMEN:
Clotted blood

Patient preparation: Fasting individual

Type:
Serum, freshly drawn from a fasting individual. It can be stored at 2 to 8 degrees for up to 72 hours. If kept longer they should be frozen. Plasma is not recommended.
CSF; centrifuge to remove possible cellular or bacterial contaminants. Analyse freshly or store in fridge.
Interfering substances:
Lipaemic specimens tend to cause light scattering. The presence of dust particles causes non-specific interference, and the use of polymer enhanced buffers.
CSF; blood contamination may produce erroneous results.

EQUIPMENT AND MATERIALS:

Equipment:
Beckman Array Nephelometer
Materials:
Beckman IgG antisera. Reorder no: 446600

Storage Requirements:
1. Antibody, ready to use, comes with the Antibody card.
2. Bring reagents to room temperature before use.
3. Store at 4 degrees until expiry date.

4. Do not mix reagents of different lot numbers.

CALIBRATION:

Standard Preparation:

NOTE:a) Each calibration Kit consists of:

1. A calibration card
2. A calibrator

They must always be used together.

b) The Calibration Procedure is the same for all the chemistries used on the Array.

I: Ready for use, comes with calibrator card which has calibrator values on it. Use only same lot numbers together.

II: Reconstitute with 1 ml distilled water, let stand for 30 minutes. Use same lot numbers for card and calibrator.

Calibration Procedure:

Calibrate on CAL I. Use Control I.

1. "Master Menu"
2. "Results recall" (F3)
3. "Clear current run" (F4) (You cannot calibrate unless all results have been deleted).
4. "Master screen"
5. "Cal Status" (F2)
6. "Select chemistries to be calibrated"
7. PRESS "Read AB cards"(F1)
8. Use the appropriate Array Antibody card (i.e the one in the reagent kits) one at a time, checking that the correct reagent lot number is used.
9. Define the cup location of the calibrator.
10. Proceed to "Cal Tray Set-up"(F2) and insert the appropriate Array Cal Cards (one at a time). ReadCal Card F2.
11. "Save cup" (F1) to store the program in memory.
12 Place 200µl of the appropriate Cal solution into the outside well of the dilution segment and place on turntable in the desired position.
13. Place all your antibody vials in the correct positions, mix and uncap. Also check that you have got sufficient antigen excess solution.
14. Press (START) to initiate calibration.
15. When the calibration is finished, the analyzer will print for each chemistry a x-value and a y-value.The two values must be the same for the calibration to be successful, if not the print out will say "CALIBRATION FAILED". The procedure must then be repeated.

QUALITY CONTROL:

I: Ready for use. Inserts have target values for different chemistries.

II: Reconstitute with 1 ml of distilled water, stand for 30 mins.

PROCEDURE- STEPWISE:

1. "Master Screen"
2. "Clear Current Run" (F4)
3. "Sample Program" (F1)
4. "Cup" : enter position 1 - 40 (ENTER)
   "Sample ID" : enter lab number (ENTER)
   "Sex" : skip
   "Age" : skip
   "Panel" enter panel number or go to individual tests using arrow keys and select. (Tests get highlighted)
5. "Save Cup" (F1)
6. Place a minimum of 150 µl of your sample in the outside well of the dilution segment and place on turntable in the desired position.
7. Gently mix your reagents, put them in the correct position and open the lids.
8. START
9. All results will be printed as the analyzer completes each test.

PRIORITY SAMPLE PROGRAMMING

Once a priority sample is entered as above, the analyzer will finish any test presently in the optics and then run the priority sample irrespective of its position on the tray. To designat a sample as a priority, simply press F3 any time before saving the cup (F1).

CONTINUOUS PROGRAMMING (ADDING-ON)

Additional cups can be programmed to an existing run by identifying the cup position and loading, (preferabley when the analyzer is finished loading the initial samples, so as not to interfere with the probe movements) otherwise press "PAUSE" and load freely and then press "START".

DAILY START-UP

The power should be switched on at all times.

1. Check the syringes on the left outside for leakages.
2. Empty the waste container.
3. Check that the buffer, diluent,RHF reaction fluid levels are high enough.
4. Prime the appropriate lines i.e
   System prime mosde 1 (lines 9 and 14) diluent and buffer.
   System prime mode 2 (lines 9 and 13) diluent and RHF fluid.
   The system will prime itself automatically at 4 hour intervals.

5. Go to the keyboard and press:
   a. Master Screen
   b. System set up
   c. Optics update
   d. Left optics (F1)

The probes align themselves, then follow the following prompts from the screen.

i. "homing" (no action)
ii. "draining" (no action)
iii. "replace flow cell with 120 cell, press (ENTER) to continue".
iv. "replace 120 cell with black cell, press (ENTER) to continue".
v. " reading scatter standard" (no action).
vi. "Replace black cell with flow cell and press (ENTER). *Verify that the stir bar is in the flow cell beforep putting it back in *.
vii. Replace the flow cell and cap then press (ENTER).

DO THE SAME FOR THE RIGHT OPTICS (F2)

GAIN ADJUST

1. The gain adjust should be between - 15% and + 15%.
2. Record the adjust % daily (should trend gradually in the negative direction).
3. The gain 1,2 and 3 results should be 120 +/- 1. Gain 4 should read "over", if not, repeat OPTICS UPDATE and if still out of limits then do OPTICS SET.
4. Optic Set is normally done once in 6 months under normal circumstances - follow instructions on the screen as for OPTICS UPDATE.
5. Once you do an OPTICS SET you will lose all calibrations.
6. The same 120 scatter cell used for OPTICS SET, should be used for daily OPTICS UPDATE, handle the cell at the top with the numbers facing the front.

If your calibrations are still valid, you are now readyto program your specimens. Calibrations are done once a week (usually on a Monday).

CALCULATIONS: None
REPORTING RESULTS: in g/l
REFERENCE RANGES: please see MATERIALS AND METHODS page 86.

APPENDIX G

	HOSP NO	PATIENTS NAME	ADMIN MNTH	WARD	WCC	RCC	HB	HCT	MCV	MCH	MCHC	PLTS	RETIC	NEUT	LYMPH	MONOS	EOS	BASOS	METAS	MYELOS	PROS	BLASTS	PARASIT	MORPH	DAYS PARA	IgG	IgM	Na	K	CL	CO2	UREA	CREAT	GLUCOSE	TP	ALBUMEN
1	105870	BULELANI IGANYE	MAR (69)	GEN	3.8	2.09	5.9	0.19	92.9	29.2	30.4	205		34	55	5	4	2	0	0	0	0	<1	T;PF		13.2/>1:80	1.0/=1:20	141	3.6	110	21	3.2	45	5	45	
2	GI115563	ANTONIO PAULINO	MAR(67)	GEN	13.3	4.39	13.1	0.4	89.8	29.9	33.2	183		73	24	3	0	0	0	0	0	0	<1	T;PF	4	17.2/>1:80	1.8/=1:20	139	3	105	22	4.6	86		78	
3	GI115155	MALEYANE JOEL	MAR (66)	GEN	4.1	5.09	15.5	0.47	92.4	30.5	33	43		69	8	3	0	0	0	0	0	0	4	T;PF	5	7.9/>1:80	.60/=1:20	135	3.4	102	18	9	136		30	10
4	GH359958	VILAKAZI EDWARD	MAR(77)	GEN	C	4.15	9.8	0.31	74.2	23.7	32	90		71	26	1	2	0	0	0	0	0	<1	T;PF	3	12.6/>1:80	5.0/>1:80	142	3.7	109	21	11.4	81		50	21
5	6859431P	MAKHUBO EDNA	MAR(99)	GEN	16.5	1.89	4.5	0.14	72.3	23.9	33.1	119		25	68	9	0	0	0	0	0	0	5	T/G; PF	7	/>1:80	/>1:80	138	3.8	105	15	4.8	40	7.5		
6	GI115375	AUNAH FLORENCE	MAR (62)	GEN	3.2	1.36	4.3	0.14	104.4	32.2	30.9	133		67	29	2	2	0	0	0	0	0	1	T;PF		13.9/>1:80	2.9/>1:80	135	4.1	106	17	2.5	62	5.9	62	36
7	114074	KOGALE ROSINAH	MAR (90)	PSYCH	4.7	2.49	7	0.21	84.8	28.4	33.4	151		50	43	7	0	0	0	0	0	0	17	T;PF	4	19.9/>1:80	3.9/>1:80	135	3.4	103	21	5.4	49		74	35
8	6807941P	KOGALE THEMBA	MAR	GEN	16.6	1.41	4.8	0.15	103.2	34.3	33.3	208	16.8	72	27	1	0	0	0	0	0	0	2.8	T;PF	4			135	3.7	105	17	1.8	37		71	34
9	112992	SOTHU JONAS	MAR (62)	ICU	5.9	3.62	10.8	0.34	93	29.9	32.2	28		83	14	3	0	0	0	0	0	0	3	T;PF	6	29.8/>1:80	1.2/>1:80	138	3.7	109	13	29.3	424	12	72	25
10	GI113003	MOJI JULIA	MAR(102)	GEN	5.6	4.44	14	0.41	93.1	31.6	33.9	82		84	13	3	0	0	0	0	0	0	12	T;PF		/>1:80	/>1:80									
11	112804	MABASO ELISA	MAR(101)	GEN	7.3	3.05	8	0.25	81.4	26.2	32.2	190		80	15	5	0	0	0	0	0	0	2	T;PF	4	/>1:80	/>1:80	135	2.9	102	20	5	97	7	70	41
12	GI011714	MLAMBO SAMSON	MAR(103)	GEN	4.8	4.23	12.7	0.4	94.2	30.2	32.1	94		49	29	3	19	0	0	0	0	0	<1	T;PF		/>1:80	/>1:80	143	3.7	104	22	4.2	83			
13	109351	SHEMBO THOMAS	MAR (64)	GEN	5.1	4.85	13.2	0.41	83.9	27.1	32.4	81		77	19	4	0	0	0	0	0	0	1	T;PF		18.1/>1:80	2.1/=1:20	138	3.2	100	19	7.1	141		74	40
14	109051	S.THOLE SIMON	MAR (65)	GEN	8.5	4.97	13.3	0.41	82.3	26.8	32.6	237		63	29	8	0	0	0	0	0	0	<1	T;PF		19.8/>1:80	2.2/=1:20	139	3.5	1.6	23	3.3	108		82	44
15	6848131P	DICKSON ZITHA	MAR (73)	ICU (+)	20.4	1.78	4.2	0.13	76.9	24.1	31.7	169		60	33	7	0	0	0	0	0	0	6	T/P;PF		18.3/>1:80	1.4/>1:80	128	4.5	94	8	3.4	66	3.9	64	36
16	109845	MOALEKI THELMA	MAR (71)	MATERNITY	5.2	2.16	5.8	0.19	82.1	27.3	33.2	36		49	47	4	0	0	0	0	0	0	12	T;PF	4	19.4/>1:80	3.4/>1:80	126	2.5	96	18	9.6	82	1.7	56	22
17	GI112278	PHAKATHI WALTON	MAR (98)	GEN	5.1	4.73	15.2	0.45	94.5	32.1	34	56		58	38	4	0	0	0	0	0	0	2.5	T;PF	5	21.6/>1:80	3.9/>1:80	130	4	102	17	6	130		73	36
18	110012	MAGUBANE ROBERT	MAR (74)	GEN	4.1	5.09	16	0.47	92.5	31.4	34	50		62	33	4	0	1	0	0	0	0	4	T;PF	5	19.9/>1:80	3.5/=1:20	135	4.3	100	24	6	88	5.6	74	35
19	111363	MTHEMBU ERNEST	MAR (91)	GEN	3.6	5.41	12.2	0.39	71.9	22.6	31.4	116		70	25	3	2	0	0	0	0	0	<1	T;PF	5	18.7/>1:80	1.5/>1:80								69	38
20	6858691P	REFOTH MABALE	MAR	GEN	18.2	4.16	12.2	0.34	80.5	29.3	36.4	65		71	23	6	0	0	0	0	0	0	7	T;PF												
21	GI112839	ZUMA MICHAEL	MAR(94)	GEN	9.2	5.78	13.7	0.42	72.8	23.6	32.7	47		64	29	7	0	0	0	0	0	0	18	T;PF/PV		20.9/>1:80	3.0/>1:80	130	3.9	97	13	11.5	158		60	28
22	6596534P	ZUMA GIRL	MAR (70)	GEN	11.8	1.62	3.9	0.12	74	24.6	33.2	154		44	47	6	0	0	1	0	0	0	16	T;PF	5	8.8/>1:80	2.4/>1:80	132	4.3	102	13	2.4	46	0.3	55	35
23	GI 10329	MOGANE JACK	MAR (93)	GEN	7.5	4.26	13.4	0.41	96.6	31.5	32.6	26		84	12	4	0	0	0	0	0	0	4	T;PF	5	16.7/>1:80	3.1/>1:80	140	304	109	17	15.6	211		64	33
24	111349	KHOZA ALEX	MAR (78)	GEN	4.4	4.93	12.9	0.39	81.2	26.8	33	62		66	30	4	0	0	0	0	0	0	2.5	T;PF		19.2/>1:80	2.3/>1:80	131	3.4	101	18	7.7	123		65	33
25	GI111694	NOKANE JEANETTE	MAR (75)	GEN	5	1.58	6.1	0.19	118.7	38.7	32.6	163		47	42	9	2	0	0	0	0	0	<1	T;PF		35.0/>1:80	2.3/>1:80	138	3.8	109	19	2	67	5.2	96	40
26	A54585	LONGME LEONARD	MAR	CLINIC																			3	T;PF												
27	111696	MOTHANE DONAH	MAR (97)	CLINIC	10.6	4.31	14	0.41	96	32.5	33.8	307		37	50	10	3	0	0	0	0	0	<1	T;PF		19.0/>1:80	2.4/>1:80	140	3.1	102	24	1.4	78	5.9	73	41
28	110787	TELE ALEXANDER	MAR (80)	CLINIC	5.7	1.66	12.8	0.41	86.5	27.4	31.6	82		24	73	2	1	0	0	0	0	0	2.5	T;PF				140	3.4	99	25	5.7	56		70	39
29	113569	MSUNYATHE JULIUS	MAR (85)	GEN	3.2	5.33	16.2	0.48	90.1	30.5	33.8	146		70	21	9	0	0	0	0	0	0	4	T;PF	3	20.5/>1:80	3.6/>1:80	136	3.9	103	20	4.9	90		68	41
30	114547	MACLOLO FRANK	MAR (79)	GEN	6.2	4.51	13.8	0.41	91.4	30.7	33.5	66											3	T;PF	3	19.1/>1:80	2.3/>1:80	133	3.3	102	18	8.6	127		64	34
31	6852761P	MOJI ALINAH	MAR (87)	GEN	3.7	3.19	9.8	0.27	84.8	27.8	32.8	76		47	48	5	0	0	0	0	0	0	5	T;PF	3	17.1/>1:80	2.8/>1:80	131	4.3	95	17	3.6	49		64	34
32	116784	SONGO ANITA	MAR(100)*	GEN	8.3	3.75	8.5	[illegible]	[illegible]	22.6	30.3	268											<1	T;PF		/>1:80	/>1:80									
33	117580	TEMBE CATHERINE	MAR	GEN	8	2.9	8.3	0.25	85.9	28.5	33.2	65											9	T;PF												

KEY:

GEN	General medical wards	Codes for clinical presentation: e.g GIT(SIGNS)/ OUTCOME(D/A)					
*( )	specimen number allocation for IFAT/Ig's	0	nil	0	ALIVE		
T	Trophozoite	1	spleen	1	DEAD		
P	Pyrexia	2	liver	2	data not available		
PV	*P.vivax*	3	spleen and liver				
PF	*P.falciparum*	4	other				
M	Male	5	data not available				
F	Female						
IFAT	indirect fluorescent antibody test						
Ig	Immunoglobulins (IgG/IgM)						

OLORULIN	TB	DB	ID	AST	ALT	GGT	ALKPHOS	AGE	M/F	PREGNANCY	TEMP	FEVER/DOORS	NIGHTSWEAT	MAL+INTPAIN	WEAKNESS	HEADACHE	RESP	CVS	GIT(SYMPT)	GIT(SIGNS)	CNS	JAUNDICE	PALLOR	TRAVEL HX	OUTCOME (PRES)	OUTCOME(DIA)	BLD TX	
21								14	M	0	1	1	1	1	1	1	1	0	6,4	3	0	2	0	1		0	0	
34								27	M	0	0	2	0	0	0	1	1	0	0	0	0	0	0	1		0	0	
14								<2	M	0	1	1	0	1	0	0	0	0	5	0	1	0	1	1		0	0	
29								20	M	0	1	2	0	1	1	1	0	0	6	0	0	1	1	1		0	0	
								<1	F	0	1	1	0	1	0	0	0	0	2	3	1	0	1	1		0	1	
28	23	10	13	15	11	8	59	18	F	4	1	1	0	1	0	1	0	0	5	3	0	0	1	1		0	1	
39								30	F	0	1	2	1	0	0	1	1	0	5	1	0	0	1	1		0	0	
37				22	30	12	156	1	M	DATA NOT AVAILABLE																2	5	
45	47	32	15	65	38	75	119	62	M	0	1	1	1	1	1	0	0	0	5	0	1	1	1	1		0	3	
								32	F	0	1	1	1	0	0	0	0	0	6,4	0	0	1	1	1		0	0	
38	8	3	5	30	29	18	77	35	F	0	1	2	1	1	0	1	0	0	6,5	0	0	1	1	1		0	0	
								47	M	0	0	2	1	1	1	1	0	0	2	0	0	0	0	1		0	0	
34								24	M	0	1	2	1	1	1	1	0	0	2	0	0	0	0	1		0	0	
38								36	M	0	1	2	1	1	1	1	0	0	6,4	1	0	0	0	1		0	0	
28	43	23	20	379	93	23	163		M	DATA NOT AVAILABLE																1	5	
34	137	88	49	111	38	8	41	27	F	3	1	2	1	1	1	1	0	0	0	3	0	1	1	1	0	0	0	
37	47	21	26	173	76	55	67	46	M	0	1	2	1	1	1	1	0	0	0	0	0	1	0	1		0	0	
39	31	14	17	43	30	35	76		M	0	1	2	1	1	0	1	0	0	0	0	0	0	0	1		0	0	
31	69	15	54	16	11	17	46	29	M	0	1	1	1	1	0	1	0	0	6	0	0	1	0	1		0	0	
								9	F	0	1	1	0	0	0	0	0	0	6,2	0	0	0	0	1		0	0	
32								46	M	0	1	1	0	1	1	0	1	0	0	3	0	0	1	1		0	0	
20	33	16	17	97	31	19	129	<1	F	0	1	1	0	1	0	0	0	0	5	3	0	0	1	1		0	0	
31	66	32	34	196	128	103	116	75	M	0	-	2	1	0	1	1	0	0	0	2	1	1	0	1		0	0	
32	33	15	18	40	19	23	116	18	M	0	1	2	1	1	1	1	0	0	6	0	0	0	1	0		0	0	
56	16	6	10	17	8	21	69	20	F	0	0	2	1	1	0	1	0	0	6;1	1	0	0	0	1		0	0	
								65	M	DATA NOT AVAILABLE																2	5	
32	16	7	9	34	23	79	84	29	F	DATA NOT AVAILABLE																2	5	
31	29	12	17	46	52	58	123		M	DATA NOT AVAILABLE																2	5	
47								29	M	0	0	2	1	1	1	0	1	0	5	0	0	0	0	1		0	0	
30								30	M	DATA NOT AVAILABLE																2	5	
30								3	F	DATA NOT AVAILABLE																2	5	
									F	DATA NOT AVAILABLE																2	5	
									F	DATA NOT AVAILABLE																2	5	

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