NEW MODALITIES IN THE DIAGNOSTICS OF MALARIA AND STRATEGIES FOR CONTROL*

Fatima Mujib Bilshees**

Abstract

Malaria is the most prevalent endemic disease in several parts of the world including Pakistan. Early diagnosis is an essential element for the control strategies of the disease. Microscopic examination of thick and thin blood films continues to be the gold standard test, other alternative and new methods for malaria diagnosis are adopted due to various reasons, which are described here.

Controlling the disease by drug treatment and other means remains unsatisfactory. Strategies for malaria control include use of impregnated bednets, screening and control of anemia, estimating hemoglobin concentration, Mefloquine prophylaxis during pregnancy and iron prophylaxis to the pregnant women. Now development of vaccination against malaria is a major research goal of malaria immunology.

Community based antimalarial programme and community health education is also important for malaria control.

Key Words

Malaria, new modalities, diagnosis, control strategies.

Introduction

It was estimated that world wide incidence of malaria is more than 100 million clinical cases and about 1 million deaths per year. Today around 300 million people are infected and between 1.5 and 2.6 million die each year.

Malaria is a major cause of health problems in several parts of the world. It is characterized by prostration associated with sudden intensification of symptoms of high fever, shaking chills, sweating, anemia and splenomegaly, with intervals between the attack being determined by the time required for the development of a new generation of the parasites in the body.

Four common species of Plasmodium are known in humans namely: *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae* which have specific morphology and symptoms

General Complications

Falciparum malaria is the malaria caused by *Plasmodium falciparum*, in which febrile paroxysm recur irregularly. It is associated with the highest levels of parasites in the blood and is the most severe form of malaria, and may be fatal. It is one most likely to be associated with pernicious symptoms, which occur as a result of plugging of capillaries with *P. falciparum* infected erythrocytes, usually confined to one organ system, including the brain, liver, adrenal gland, gastrointestinal tract, kidney and lungs. It is also called malignant tertian malaria, pernicious malaria, and subtertian malaria.

Cerebral malaria is a severe and often fatal complication of falciparum malaria mainly involves brain. It is characterized either by the gradual onset of headache, confusion, and psychotic manifestations lapsing into delirium and coma, or by sudden onset of an abrupt rise in temperature sustained at high levels, convulsions and...
coma.

A complication of falciparum malaria mainly involving the liver is known as bilious remittent malaria. It is characterized by continuous vomiting, epigastric and hepatic tenderness, marked jaundice and high remittent fever. Falciparum malaria in which hemorrhage is a prominent symptom is called hemorrhagic malaria.

A severe complication of falciparum malaria is manifested by shock, syncope, peripheral vascular failure, hypotension, cold, clammy skin, gastrointestinal symptoms, diarrhea, and vomiting is known as algid malaria is sometimes followed by coma and death.

It is know that \textit{P. falciparum} invade human red blood cells and immediately begin making significant alterations to the structure of the erythrocytes. These alterations facilitate the movement of nutrients into, and waste products and parasite derived proteins out of the cell to meet the needs of the growing parasite. The knob-like protrusion described at the \textit{P. falciparum} infected cell surface is the parasite derived proteins. The parasite modifies the erythrocyte membrane itself and changes its permeability\textsuperscript{34}.

Vivax malaria is caused by \textit{P. vivax}. In this form of malaria the febrile paroxysms occur daily, due to simultaneous infections with two broods of \textit{P. vivax} which complete their 42 to 47 hours cycle on alternate days. It is less severe than falciparum malaria. In this relapses are most likely to occur because of hypnozoites forms in the liver. In vivax malaria the febrile paroxysm recur after every other day, but may recur daily. In a stained blood film the infected red cells often appear enlarged. As the parasite tends to infect younger erythrocytes. This is also called as benign tertian malaria\textsuperscript{34}.

Ovale malaria is caused by \textit{Plasmodium ovale}. It is clinically similar to but milder than vivax malaria, and is frequently found in conjunction with infections due to \textit{P. falciparum}. The infected erythrocytes assume an oblong or oval shape on a stained blood film. It is also known as tertian malaria. The febrile paroxysm occur every 42-47 hours, or every third day counting the day of occurrence as the first day of each cycle. \textit{P. malariae}, requires 72 hours for completion of each asexual cycle in the erythrocytes. It is the mildest and most chronic of all human malaria infections\textsuperscript{34}.

Infection with \textit{P. Vivax}, \textit{P. ovale} and \textit{P. malariae} generally are not life threatening but infections caused by \textit{P. falciparum} may produce severe illness and death if not promptly diagnosed and appropriately treated. The risk factors that predispose to complications are high density parasitemia, pregnancy and lack of immunity to malaria. In this malaria up to 50% red cells are infected\textsuperscript{34}.

New Modalities in the Diagnostics

The initial symptoms and signs of malaria are not pathognomic, fever is an invariable finding, but in the early stages of the illness, fever may alternate with afebrile periods when the patient feels relatively better. Depending on the patients history of travel, exposure to infectious diseases and other illness, the differential diagnosis may include a wide variety of infectious agents.

Braendli\textsuperscript{2} suggested that malaria must be included in the differential diagnosis of all febrile patients. This disease is classified as complicated, or uncomplicated, according to clinical finding that is cerebral malaria, generalized convulsion, pulmonary edema, severe anemia, bleeding. Laboratory results must also be taken into account. \textit{P. vivax}, \textit{P. ovale} and \textit{P. malariae} cause uncomplicated disease, \textit{P. falciparum} may be uncomplicated or complicated. In complicated falciparum malaria there is usually a risk of fatal consequences.

Confirmation of infection with \textit{Plasmodium} species depends on finding the parasites in the peripheral blood smear. Accurate species diagnosis and density of parasitemia depends on meticulous examination of blood smears. It is the basis of appropriate therapy and for an initial estimate of the risk of complications including death. When the possibility of malaria exists, prompt and accurate examination of blood films is essential. Microscopic examination of thick and thin blood film continues to be the gold standard test, other alternative methods for malaria diagnosis are adopted due to various reasons.

Blood smears should be obtained when the diagnosis is considered and should be repeated every 6-12 hours until the diagnosis is confirmed or excluded. Collection of blood
smears at the time of accurate symptoms does not necessarily enhance the sensitivity of extermination. During the fever chill paroxysm, the majority of parasites tend to be young trophozoites, therefore, at this stage specific diagnosis is most difficult.

Symptoms of malaria even with very low density parasitemia may develop in non immune persons, therefore thick blood smear is essential for maximal sensitivity. Thin blood films should be prepared at the same time to permit detailed assessment of the morphology of red blood cells and parasites for accurate determination of the infecting species.

The Giemsa stain is preferred for examination of blood smears because it yields optimal coloration of the young ring trophozoites and provides the morphologic details of parasites and infected erythrocytes necessary for species diagnosis. No single or composite hematologic or biochemical tests will confirm the diagnosis of malaria. The white blood cell count is generally either normal or decreased, the differential white blood count similarly, is normal or shifted towards mononuclear series.

When the density of parasitemia exceeds 5% RBCs infected and the hematocrit falls below 20% the patient is at substantial risk of complication and death. Cerebral malaria may occur any time during the course of disease, beginning most often with disturbance of consciousness that may progress to coma.

Persons with malaria usually become dehydrated because of increased insensible loss of water and decreased intake of liquids. Enlargement of spleen is common in patients with malaria and may cause left upper quadrant tenderness and pain. Splenic puncture is a rare complication that is most commonly associated with vivax malaria. It may occur spontaneously or result from minor trauma such as splenic palpation. It is a diagnosis that should be considered in a patient with malaria who has sudden cardiovascular collapse.

Prompt recognition, aggressive blood replacement and expeditious surgical intervention are life saving. Serologic test for malaria are available, but none are useful for diagnosis of acute infections. The indirect fluorescent antibody test (IFA) is most commonly used and measures antibodies when they become detectable in the second week after a primary parasitemia. Antibodies may persist for several years; the test is well suited for recording the malaria experience of population group.

The ParaSight-F is a new diagnostic test for *Plasmodium falciparum* infections. It is based on the detection of a trophozoite derived antigen, the histidine rich protein 2 (HRP2). The ParaSight-F test does not allow following up the efficacy of treatment, identifying other *Plasmodium* species but this test is easy to perform and has good sensitivity and specificity. It is a useful tool in emergent context, in cases of parasitemia lower than the thin blood film threshold, and in cases morphologically difficult to explain. The ParaSight-F test is known to have 86.4% sensitivity and 100% specificity. ParaSight-F test can be performed with minimal training and may be specially useful in areas where *P. falciparum* is the predominant malaria species, in epidemic malaria region, and where skilled microscopy is not readily available.

In some countries like Sri Lanka ParaSight-F dipstick test is a routine diagnostic method for malaria. The ParaSight F test reading correlates significantly and positively with the intensity of clinical disease of patients but not with their peripheral parasitemia, which indicates that it can be a more accurate measure of true parasite load than microscopy which detects only parasites which are in the peripheral blood and not those in deep organs.

Rapid diagnosis of *P. falciparum* malaria remains one of the main limitations to prompt treatment. Diagnosis based on clinical symptoms alone is unreliable, specially in areas of seasonal transmission. It was found out by several authors that ParaSight-F test reduces mistreatment for malaria, relative to clinical diagnosis, by up to 81%, specially in hypoendemic region.

Para Sight F test can not replace microscopic techniques by which species can be identified. Although in endemic areas the test seems to be very promising indicating presence of malarial parasite. According to published field studies it gives good results and easy to perform and it is performed quickly i.e. ten tests in 20 minutes.

Measurements of the *P. falciparum* rich protein 2 (PFHRP2) antigen in plasma could provide an alternative approach to the assessment of parasite biomass, and thus prognosis, in severe malaria, and that this could be done simply by using the currently available dipsticks. Dipstick test represents a simple and accurate test for the diagnosis of *Plasmodium falciparum* infection in returned travellers. According to the authors the ICT-Malaria p.f test is
simplest and easier to use than the ParaSight-F test. Although both tests provide results within ten minutes. The ICT Malaria P. f test is specially suited for infield use.

Quantitative Buffy Coat method (QBC) is based on the principle of the centrifuged hematocrit. The QBC malaria test is simple, and sensitive providing rapid and accurate results. This method is useful for early detection and is 100 times more sensitive for low level infections than the thick film method. In clinical studies in malarial areas, it has proven to be over eight times more sensitive. The health care workers can learn to process and interpret QBC tubes-site within a day.

Tanspradist and coworkers have shown that Quantitative Buffy Coat detected more cases with *P. falciparum* infection and smaller number of cases with *P. vivax* infection. It suggests that QBC is a better indicator for *P. falciparum* infections. They also have shown that QBC and thick blood film (TBF) results correlated with agreement of 95.8% for *P. falciparum*. Similarly both monoclonal antibody ELISA and TBF based also correlated with agreement of 95.9%.

A recently described diagnostic test for malaria is the optimal Assay. It is an immunochromatographic test that utilizes a panel of monoclonal antibodies that binds to the active PLDH protein. The PLDH (Plasmodium Lactate Dehydrogenase) is bound to a monoclonal antibody that recognizes PLDH from all four species of malarial parasites. The monoclonal antibody is conjugated to small coloured beads. Bound PLDH attached to the coloured beads is then passed over a dipstick that contains two test lines and a control line. Results can be obtained within 10-15 minutes. It is supposed to be both specific and sensitive. This test is rapid and is suitable for use in both hospital and field clinics.

DNA detection of the malaria parasite using immunomagnetic separation in combination with the PCR and colorimetric analysis is a possible model for large scale testing of malaria with an increased sensitivity compared with conventional methods.

*Plasmodium falciparum* Glutamate dehydrogenase antigen GDH (NADP+), obtained by affinity chromatography in Elisa assays is also demonstrated to be useful in diagnosis of malaria. This is done by testing IgG antibodies against GDH (NADP+) from sera of hyperimmune patients. This antigen is soluble has high specificity, and is a potent immunogen and is thermoresistant.

Diagnosis of acute malaria, mainly for screening blood donors in endemic area has also been suggested. An anti-40-KDA polypeptide antibody response associated with active or recent infection was identified and IgG/IgM ratio of antibodies to blood stage *P. falciparum* antigen was studied 40 KDA polypeptide may represent a powerful tool for the diagnosis of acute malaria. Use of the IgG/IgM ratio values did not differentiate acute from past infections.

Incidence of infection with *P. falciparum* among international travellers can be assessed. Circumsporozoite (CS) antibodies, indicating plasmodial infection but not necessarily development of disease, have been shown to be reliable indicators of transmission in endemic area. The prevalence of CS antibodies are investigated by an ELISA test system in a selected population returning from areas endemic for malaria.

Transplacental passage of *P. falciparum* can be confirmed by detection of parasitemia in the peripheral blood of newborns within 7 days of birth. But seroconversion of newborns by Indirect Florescent Technique with *P. falciparum* IgM specific conjugate indicate more sensitivity.

**Strategies for Control**

Elimination of malaria infection is very difficult specially in Pakistan where there are great opportunities for breeding of mosquitoes and poor communities cannot afford the expenses for protective measures. Only on Government level control programmes may have some success. Lack of awareness is another problem which is directly linked with illiteracy. Many approaches to malaria control are involved during the chances of infection. Awareness of cause of disease is essential, community based education campaigns are needed to increase the possibility of acceptance and support of control programmes.

An intervention that can effectively reduce malaria infection in primigravidae could have a major impact on the health of the women and their infants.

Impregnated bed nets for personal protection can be used. Permethrin impregnated bed nets for personal protection against malaria appears good despite people's lack of previous experience. A single treatment of the nets with
permethrin can remain protective throughout the 6 months transmission period\textsuperscript{35}.

National impregnated bed net programme (NIBP) has been introduced in Gambia in 1992. The insecticide treatment of bed nets into half of the primary health care villages has been carried out. Despite of the low use of insecticide-treated bed nets by Gambian primigravidae, the NIBP had some impact on the outcome of pregnancy, particularly on the percentage of premature babies, and this was probably due to decreased risk of malaria infection achieved during the period\textsuperscript{36}.

Bed nets are useful for the prevention of malaria and anemia in pregnancy. Clinical trials have revealed that the overall effect of bet nets on potent paracitaemia was marginal and they were associated with a significant reduction in maternal malaria associated anemia\textsuperscript{26}.

Screening of anaemia in malaria control is a practical approach. It can be done by the use of haemoglobin colour scale which is developed by WHO. It is now possible to estimate haemoglobin levels, simply, cheaply, easily and safely\textsuperscript{27}. The potential and practical value of this scale in malaria control programmes was demonstrated in a small, preliminary trial in 1995.

Estimating haemoglobin concentration from a drop of blood by means of a colour scale is supposed to be a reliable screening method for detecting anemia specially diagnosing severe malaria. In malaria endemic areas, the maintenance of low parasite density appears crucial to the survival of infants\textsuperscript{28}.

Initial trials have revealed that Mefloquine prophylaxis prevents malaria during pregnancy. Mefloquine is safe and effective for anti malarial prophylaxis in second half of pregnancy\textsuperscript{29}.

Pregnancy morbidity and adverse birth outcomes party reflex poor management of malaria during pregnancy. Strategies to decrease the prevalence of anemia in young children should include chemoprophylaxis for pregnant women, prevention of acquired malaria infection in both pregnancy and infancy, and prevention of nutritional iron deficiency\textsuperscript{30}.

Malaria can cause anemia in pregnant women which can be the major cause of maternal mortality specially during the first pregnancy. It can also cause fetal anemia which frequently results in retarded intrauterine growth and low birth weight. Therefore, prophylactic treatment with antimalarial drugs during pregnancy is recommended in endemic areas\textsuperscript{31}.

In a clinical trial it has been demonstrated that the birth weight of children can be increased by iron prophylaxis to the pregnant women given by traditional birth attendants (TBAs). This can produce significant beneficial effects on the health of the mother and has a potential for reducing perinatal mortality and increasing birth weight\textsuperscript{32}.

Strategies to reduce anemia in infants must address \textit{P. falciparum}, both in pregnant women during pregnancy and in the first few months of life as pregnant women and young children commonly suffer with anemia, specially in rural areas\textsuperscript{33}. It is also emphasized by several workers that strategies to reduce the prevalence of anemia in young children should include chemoprophylaxis for pregnant women.

Malaria plays a major etiological role in anemia in infants particularly severe anemia and this justifies the use of anti malarial chemoprophylaxis in infants\textsuperscript{34}. Iron supplementation improves appetite and growth in children\textsuperscript{35}.

Interventions which lower parasite densities in areas of intense transmission reduce the development of severe malarial anemia and thus malarial related mortality and morbidity in infants\textsuperscript{36}.

\textit{Plasmodium} antigenic diversitiy is a major problem towards the development of an effective malaria vaccine\textsuperscript{37}.

According to some authors quantitative study of Cytotoxic T Lymphocytes (CTL) specific for \textit{Plasmodium} species or viral pathogen in humans provides a basis for a multiepitope approach to malaria vaccine\textsuperscript{38}.

Antibodies to gametocyte antigen (Pfs2400) have been shown to inhibit the development of \textit{P falciparum} in anopheline and this antigen is therefore a candidate for a transmission - blocking vaccine. There is a relatively short persistence of anti - pfs2400 repeat peptide antibodies under natural field conditions\textsuperscript{39}. It is suggested by the authors that gametocyte antigen booster before a high transmission period might contribute towards malaria incidence by eliciting a partially effective antibody response\textsuperscript{40}. 
A vaccine has also been developed based on the circumsporozoite protein of *P. falciparum* which incorporates adjuvants selected to enhance the immune response. This is a recombinant vaccine based on fusion of the circumsporozoite protein, HBs Ag plus a potent adjuvant. This can protect against experimental challenge with *P. falciparum*. Field trials are indicated for this new vaccine\(^1\). The antigen consists of a hybrid in which the circumsporozoite protein fused to HB surface antigen (HBs AG) is expressed together with unfused HBs AG.

There is another view about the malaria vaccine development which suggests that an appropriately designed recombinant SERA produced from a synthetic gene in *Escherichia coli* may be an effective component of a candidate malaria vaccine\(^2\).

**Conclusion**

Severe malaria is a major cause of childhood death in Pakistan and other tropical countries. Effective management relies on rapid and correct diagnosis, prompt administration of schizonticidal drugs, careful fluid balance, prevention of convulsions and early recognition of complications as has been suggested by several authors. Intervention to lower parasite densities can reduce the development of severe malarial anemia and in turn reduces the malarial related mortality and morbidity in infants. Special attention should be given to prevent pregnancy morbidity. Pregnancy morbidity and adverse birth outcome can occur due to poor management of malaria during pregnancy. Vector control is also important, use of bed nets can help avoiding the bites of mosquitoes. Community based antimalarial programmes and community health education is important in controlling the disease.

**References**


