



Review Article

MALARIA: MINI REVIEW ON DISEASE AND DIAGNOSTIC CHALLENGES

Shalu Mengi^{1*}, Varsha A Singh², Prakriti Vohra³ and Natasha Sawhney⁴

*Corresponding Author: **Shalu Mengi** Shalumengi80@yahoo.co.in

Malaria is a serious disease which has high rate of morbidity and mortality if left undiagnosed. The World Health Organization (WHO) estimates 300-500 million malaria cases annually. Therefore, early and rapid diagnosis is mandatory to detect and treat it. The current WHO recommendations for diagnosis are prompt parasitological confirmation by microscopy or alternatively by Rapid Diagnostic Tests (RDTs).

Keywords: Acridine orange, Diagnosis, Malaria, RDT

INTRODUCTION

It has been more than 125 years since Charles Louis Alphones Laveran, a French scientist, discovered the malaria parasite, yet even today malaria as a disease continues to be the world's foremost tropical disease which kills more people than any other disease except tuberculosis (Sharma *et al.*, 2007). The rapid expansion of the resistance to what were once effective anti-malarial drugs has made the battle against malaria even more urgent as we enter the twenty first century (Mugisha and Arinaitwe, 2003).

Malaria not only kills but also imposes a great socioeconomic burden on humanity and with six other diseases (diarrhoea, HIV/AIDS,

tuberculosis, measles, hepatitis B, and pneumonia), account for 85% of global infectious disease burden. It afflicts more than 90 countries and territories in the tropical and subtropical regions and almost one half of them are in Africa (South of Sahara).

The World Health Organization (WHO) estimates 300-500 million malaria cases annually with 90% of these being in Africa. In India about 27% of the population lives in malarial high transmission areas and about 58% of it in low transmission areas. The most affected geographical areas are the northeastern states along with the states of Chhattisgarh, Jharkhand, Madhya Pradesh, Orissa, Andhra Pradesh,

¹ Assistant professor, Department of Microbiology, MMU, Solan.

² Professor and head, Department of Microbiology, MMIMSR, Mullana, Ambala.

³ Assistant Professor, Department of microbiology, SHKM,GMC, Nalhar, Mewat.

⁴ Post graduate student, Department of microbiology,MMIMSR, Mullana, Ambala.

Maharashtra, Gujrat, Rajasthan, West Bengal and Karnataka (Park, 2011). *Plasmodium falciparum* predominates in Africa, New Guinea, and Haiti; *Plasmodium vivax* is more common in central America and Indian subcontinent.

Malaria is one of the major public health challenges, subverting the development in the poorest countries in the world. Factors such as the deterioration of health systems, growing drug and insecticide resistance, failure of water management and also, land-use, and climatic changes are hypothesized to influence the emergence of malaria. Socio demographic factors such as ethnic groups, parent's education and occupation, use of protective measures, and the living standard of the family are suggested to be important risk factors for malaria and malaria epidemic.

The impact of socioeconomic factors, namely the family's financial situation, is difficult to assess due to the lack of standardized economic data of income and tax (Krefis et al., 2010).

In India, the epidemiology of malaria is complex because of geo-ecological diversity, multi-ethnicity, and wide distribution of the nine anopheline vectors transmitting three *Plasmodial* species:

- i. *P. falciparum*;
- ii. *P. vivax*; and
- iii. *P. malariae*.

Anopheles culicifacies is widely distributed and is the principal vector of rural malaria, *An. stephensi* is the primary urban vector, *An. fluviatilis* is a vector in the hills and foothills, and *An. Minimus*, *An. Nivipes*, *An. philippinensis*, and *An. dirus* are vectors in the northeast and *An. sundaicus* is restricted to Andaman and Nicobar

islands. *An. annularis* and *An. varuna* are secondary vectors with wide distribution (Kumar et al., 2007).

The strategic approaches to malaria control fall into two major areas.

- Prevention; and
- Case Management

Taken together, these strategies work against both the transmission of the parasite from mosquito vector to humans (and from humans to mosquitoes) and the development of illness and severe disease in humans. The overarching policy and strategy for vector control is "universal coverage with effective vector control". The two most powerful and most broadly applied interventions are Long-Lasting Insecticide-treated mosquito Nets (LLIN) and Indoor Residual Spraying (IRS). These interventions work by reducing the lifespan of female mosquitoes (so that they do not survive long enough to transmit the parasite) and by reducing human-vector contact. In some specific settings and circumstances, these core interventions may be complemented by other methods, such as larval source control including environmental management (World Malaria Report, 2010).

Syndromic approach is unreliable because the symptoms of malaria are non-specific, overlapping with other febrile diseases resulting in over-diagnosis of malaria, over-prescription of antimalarial drugs, under-diagnosis and inappropriate treatment of non-malarial febrile illnesses.⁸ Thus, a diagnosis of malaria infection based on clinical decision-making alone is unreliable and, if possible, should be supported and verified with a laboratory-based confirmatory test (Endeshaw et al., 2010).

Malaria is frequently over-diagnosed and

results in failure to treat other life-threatening conditions; invasive bacterial infection being the most commonly identified. Importantly, there is worryingly few data on the relationships and consequences of HIV or malnutrition among children with severe malaria. Survival in severely ill children with or without malaria depends on structured, early assessment and supportive management with resuscitation, oxygen, fluids, blood, glucose and anticonvulsants. These are largely independent of underlying aetiology (Gwer et al., 2007).

The overdiagnosis of malaria coexists with underdiagnosis due to the lack of diagnostic laboratories, with the result that antimalarial drugs are given to people who do not need them and are not given to some of those who do (Nkrumah et al., 2010).

For many years, severe malaria was pictured as essentially two major syndromes, with relatively simple underlying pathogenic processes:

- i. Severe anaemia caused by the destruction of Red Blood Cells (RBCs).
- ii. Cerebral malaria caused by obstruction of small vessels of the brain by sequestered parasites.

A major change in recent years has been the re-recognition that severe malaria is a complex multisystem disorder with many similarities to sepsis syndromes. At the clinical level, this is evident in the recognition of metabolic acidosis as the strongest predictor of death in severe malaria (Macintosh et al., 2004).

Misdiagnosis of malaria is a frequent mistake that can have deadly consequences. In resourcerich populations, malaria is uncommon

and therefore may not be recognized by clinicians who have insufficient training in tropical diseases, or by laboratory technicians who have insufficient experience interpreting blood smears. In these cases, the non-immune victim, typically a recently returned traveller is at great risk of severe disease and death if infected with *P. falciparum*. In resource poor countries where malaria is a major scourge, patients suffer the opposite problem: clinicians tend to diagnose many if not most febrile illnesses as malaria and often microscopy is not available for confirmation (Duffy and Fried, 2005).

(In malaria patients, prompt and accurate diagnosis and treatment with appropriate anti-malaria drugs is the most important strategy for effective case management.⁹ Failure to diagnose malaria correctly can lead to omission of a drug, when a drug is required, administration of a drug when no drug is required or administration of an ineffective drug. Non-rational drug use, in turn, can promote drug resistance (McKenzie et al., 2003).

The current WHO recommendations for diagnosis are prompt parasitological confirmation by microscopy or alternatively by Rapid Diagnostic Tests (RDTs) in all totems suspected malaria before treatment is started. Treatment solely on the eta of clinical suspicion should only be considered when a parasitological diagnosis to not accessible (McKenzie et al., 2003; Batwala et al., 2010).

Visual inspection of blood smears by light microscopy has been the standard diagnostic test for malaria for more than a century) (Omeara et al., 2005). Density is usually assessed by thick films, either by counting parasites per microscope field, or by counting parasites per hundred white

blood cells. Thick films contain several layers of red cells, whereas thin films contain a single layer of spread red cells. Thus, for a fixed number of microscope fields, thick films allow the microscopist to examine a larger number of red cells for the presence of parasites, and low parasitacmia can be more readily identified by thick film. Thin films are preferred to examine the morphology of parasites and determine species (Bejon et al., 2006).

In resource-poor areas, microscopic diagnosis has been shown to be insensitive and nonspecific, especially when parasitemias are low or mixed infections are present. In addition, in patients with *P. falciparum* malaria, sometimes the parasites can be sequestered and are not present in peripheral blood, so can be missed. Besides these, majority of malaria cases occur in rural areas where there is little or no access to reference laboratories and in many areas microscopy is not available (Chayani et al., 2004).

One hundred years ago, Giemsa stain was employed for the first time for malaria diagnosis. Giemsa staining continues to be the method of choice in most malarious countries, although, in the recent past, several alternatives have been developed that exhibit some advantages. Considerable progress has been made with fluorescent dyes, particularly with Acridine Orange (AO) (Keizer et al., 2002). Fluorochrome stains compromise a group of acid and basic dyes that are excited to fluorescence by light near the ultraviolet spectrum. The fluorochrome AO is capable of multicolored fluorescence. This metachromasia depends upon its binding with nucleic acids. DNA bound with AO fluoresce bright yellow or apple green while RNA fluoresces orange. This feature permits differential

fluorescence of parasites and leukocytes since the RNA of the cytoplasm of the malaria parasite at the prescribed pH and concentration of AO is more intensely stained than that of the leukocytes (Sodeman, 1970).

Detection of malaria parasites by AO staining and fluorescence microscopy has several theoretical advantages, but the high cost of a fluorescence microscope has limited its potential as a replacement for Giemsa staining in developing countries. In an attempt to address this problem 2 variations of the technique have recently been developed:

- 1) Quantitative Buffy Coat (QBC) – In this examination of centrifuged blood in AO-coated capillary tubes using a specially adopted objective lens on a conventional microscope is done; are detected in the buffy coat layer between the red cells parasites and plasma.
- 2) Detection of parasites in thin blood smears stained with AO; 2 interference filters are incorporated into a standard microscope using light from a 50 W halogen bulb, or sunlight, to select a wavelength suitable to produce fluorescence from AO-stained nucleic acids (Lowe et al., 1996).

Concentration of malaria parasite – infected RBCs by centrifugation, coupled with staining with AO and fluorescence microscopy (QBC) System; has been reported to be easier to use, more sensitive, and faster in the detection of parasites (Lema et al., 1999).

The most notable advantage of the AO method over Giemsa staining is its promptness; results are readily available within 3-10 min, whereas Giemsa staining may take 4-5 min or even longer (Keizer et al., 2002).

This method allows the use of low magnification and permits rapid scanning of large areas (Htut et al., 2002). However, QBC method requires specially prepared, disposable tubes coated with AO and anticoagulants, a microhematocrit centrifuge, and the use of a microscope with an ultraviolet lens. Problems of breakage of QBC tubes during centrifugation have been noted. In addition, the examiners reported that the shadow of the fibre optic cable and the vibration of the lamp fan interfered with vision. There was difficulty in recognizing parasites using this technique; other structures that fluoresce, e.g., Howell-Jolly bodies (nuclear remnants), may have been mistaken for parasites. Furthermore, quantifying parasitemias using this method was not possible. Young rings of *P. falciparum* may be missed by staining with AO, and this problem has been reported to be serious when the parasite concentration is low. In addition, in a small laboratory with only one microscope, where number of different samples are being examined, microscope attachments are unnecessarily cumbersome (Lema et al., 1999).

The urgency and importance of obtaining results quickly from the examination of blood samples from patients with suspected acute malaria is now made possible with the introduction of Rapid Diagnostic Tests (RDTs) (Singh et al., 2005).

RDTs for malaria were developed in the early 1990's, and welcomed with great enthusiasm as a tool to improve malaria diagnosis (Laurent et al., 2010).

RDTs are handheld cassettes detecting Plasmodium parasites by an antibody-antigen reaction (Malha et al., 2011). RDT is a device that detects malaria antigen in a small amount of

blood, usually 5-15 µL, by Immunochromatographic (ICT) assay with monoclonal antibodies directed against the target parasite antigen and impregnated on a test strip. The result, usually a colored test line, is obtained in 5-20 min (Wongsrichanalai et al., 2007).

Using RDTs for diagnosis at the community level will shorten the time between onset of symptoms and initiation of appropriate treatment, leading to less suffering by the infected individual and less potential to spread the parasite to others when the infected individual is bitten by more anopheline mosquitoes. Malaria antigens currently targeted by RDT are Histidine rich protein 2 (HRP2), Plasmodium lactate dehydrogenase (pLDH), and Plasmodium aldolase (Kyabayinze et al., 2007).

HRP2 is a water-soluble protein produced by asexual stages and young gametocytes of *P. falciparum*. It is expressed on the RBC membrane surface, and because of its abundance in *P. falciparum*, it was the first antigen to be used to develop an RDT for its detection.

An alternative type of RDT detects the enzyme pLDH which is produced by all four human Plasmodium species. pLDH, an enzyme found in the glycolytic pathway of the malaria parasite, is produced by sexual and asexual stages of the parasite. Different isomers of pLDH for each of the four *Plasmodium* spp. infecting humans exist, and their detection constitutes a second approach to RDT development (Moody, 2002).

Although HRP2 based tests are generally more sensitive than pLDH based tests, the relatively low level of specificity in diagnosing clinical malaria of HRP2-based tests is a cause for concern. This reflects the fact that HRP2 can

persist in the blood stream for several weeks, resulting in high false positive error rates among patients with cleared parasitaemia who seek treatment for illnesses other than malaria.

A high number of false positives can compromise the cost-effectiveness of these (Abeku et al., 2008).

However, Pf-pLDH based RDTs have advantages over the HRP2 based RDTs, such as the rapid clearance of pLDH after successful treatment, the absence of the Prozone effect and the fact that recently observed 1-IRP2 gene deletions impede detection of *P. falciparum* by HRP2 based RDTs (Mlitha et al., 2011).

The main benefit of rapid tests is that they are quick to perform and easy to interpret, and that they require no electricity equipment or laboratory facilities. These RDTs, however, have a number of important limitations, including suboptimal sensitivity at low parasite densities, an inability to accurately identify parasites to the species level or to quantify infection density, and a higher unit cost relative to microscopy.

Despite these limitations, RDTs are a potentially useful adjunct to microscopy, particularly where expertise is limited, and have been adopted in laboratories in many countries where infection is not endemic (Playford and Walker, 2002).

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