REVIEW ARTICLE

A Synopsis of Current Malaria Diagnosis Trends

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ABSTRACT

Malaria has remained a major cause of morbidity and mortality in the under developed and developing countries of the tropical and sub-tropical regions of the world. Globally 3.3 billion people live in areas where malaria exists, affecting 300-500 million people annually and it is estimated to be killing approximately 1-3 million people each year and 90% of these mortalities occur in African children especially in sub Saharan Africa. Currently, although several control methods are beginning to result in downward trends in incidence in some countries, the gross number of malaria cases is still on the increase due to several factors including poor and ineffective diagnosis. Prompt and effective diagnosis is essential for the management and control of malaria. Over the years evidence has shown that traditional methods for diagnosing malaria remain problematic with a number of limitations. In this synoptic review an update of malaria diagnosis is presented and discussed highlighting the limitations and difficulties of both clinical (symptoms/ clinical signs-based) and laboratory (parasite-based) diagnosis of malaria. Enhancement of accurate malaria diagnosis is now more imperative than ever not only in the background of the current new era of malaria treatment with relatively expensive artemisinin-based combination therapies (ACTs), but more so in the heightened global campaign to effectively control, manage and possibly eradicate malaria from the face of the globe.

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INTRODUCTION

The onslaught of the human race by malaria over the centuries is well documented in history and it appears the scourge has been menacing mankind from time immemorial.[1-3] The oldest fossil puts the age of *Plasmodium* species at 2-10 million years and evolution of the most virulent malaria parasite, *Plasmodium falciparum*, is estimated to be about 100 000 years ago. This is at least more than 100 times older than the *Broken Hill* man.[1-4] The oldest classical and systematic clinical description of the disease was by the ancient father of medicine, Hippocrates, who between 460-377 B.C accurately gave a description of the quartian nature of the malaria fever. [5] Various holy scriptures including the Hindu scriptures[6-7] also give detailed description of malaria episodes in ancient times suggesting that, indeed, this scourge has been menacing mankind from time immemorial. Interestingly, while similar ancient scourges, such as small pox and plagues have been eradicated or at least controlled to insignificant levels, malaria continues to affect human civilization by hundreds of millions in number of people being affected. It is endemic in the developing world with 3.3 billion of the world's population being at risk of the disease

every year. It is estimated that annually 300-500 million people suffer from malaria and 1-3 million deaths, world wide, are attributable to malaria and 90% of these deaths occur in African children especially in sub Saharan Africa.[8] In Zambia malaria has recently been estimated to be causing 4 million clinical episodes and about 8000 deaths annually.[9]

Indeed, although various control methods are beginning to result in downward trends in incidences in some countries[10], the gross number of malaria cases is still on the increase due to several factors including poor and ineffective diagnosis.[11] Cumulative research evidence over the past years has indicated that effective diagnosis reduces both morbidity and mortality from malaria.[12-22] Hence, currently, there is heightened need to reinforce practical malaria diagnostics world-wide for effective control and management of malaria. In this synopsis an update of malaria diagnosis is presented and discussed highlighting the limitations and difficulties of both clinical (symptoms and clinical signs-based) and laboratory (parasite-based) diagnosis of malaria. Enhancement of accurate malaria diagnosis is now more imperative than ever not only in the background of the current new era of malaria treatment with relative expensive ACTs, but more so in the heightened global campaign to effectively control, manage and possibly eradicate malaria from the face of the globe.

CLINICAL DIAGNOSIS

Clinical diagnosis of malaria is based on patient's signs and symptoms from history and physical evaluation. It is the least expensive of all methods of malaria diagnosis and is widely practiced.[13 ,24] However, clinical diagnosis of malaria is at all times quite challenging and has various limitations due to indistinguishable nature of the disease from other illnesses with similar signs and symptoms, such as pneumonia, sepsis, the human immunodeficiency virus (HIV) infection, influenza, etc.[24-31] Indeed though clinical diagnosis has sensitivity as high as 100% its specificity is quite low ranging from 0-9%.[32-35] This non-specific nature of malaria symptoms and signs entails that in malaria-endemic areas, there is over-diagnosis leading to over-treatment of malaria and non-treatment of other illnesses (see Figure 1, for illustrative case).

Figure 1. A 13 year old Child Presumed to have malaria Black water fever.

A 13 year old boy was referred to University Teaching Hospital (UTH) on 16th July 2009 after an empirical treatment with Fansidar for malaria at a peripheral clinic that was presumed to have complicated into Blackwater fever (Note his haematuric urine sample). Subsequent investigations and clinical evaluation at UTH revealed the boy to be G6PD deficient with negative malaria parasite slide as well as a negative RDT.

Meanwhile, on the other hand, there is misdiagnosis in non-endemic areas. Thus, reliance on clinical diagnosis alone results in a number of problems as demonstrated by many reports[36-42] including; i) over diagnosis of malaria, ii) unnecessary morbidity and mortality as other illnesses end up being under diagnosed and under treated, iii) over treatment and needless wastage of health resources being spent on non-malaria cases, iv) inappropriate prescription of anti-malarials contributing to anti-malarial drug resistance, and (v) neglecting treatment of other

clinical conditions with similar symptoms. It is thus imperative that clinical diagnosis should not be solely relied on but be enhanced by combining it with laboratory based diagnosis.

LABORATORY DIAGNOSIS

Currently there is a range of different laboratory techniques to confirm and diagnose malaria including the traditional microscopy techniques, rapid malaria diagnostic tests (RDTs) and molecular techniques such as the polymerase chain reaction (PCR). Most of these are being certified by the World Health Organization (WHO) for wider routine usage.[43] Overall, and as evidenced by research locally[44] and elsewhere[45-47] laboratory or parasite-based malaria diagnosis is more cost-effective compared to clinical diagnosis in that it ensures appropriate drug use and, if prudently utilized, reduces malaria and other disease related morbidity. However, like clinical diagnosis, laboratory diagnostic techniques on their own have limitations with regard to; i) sensitivity, ii) specificity, iii) accuracy, iv) precision, v) time consumed, vi) cost-effectiveness, vii) labor intensiveness, viii) the need for skilled microscopists, and ix) the problem of inexperienced health workers (both technicians and clinicians).

Microscopy

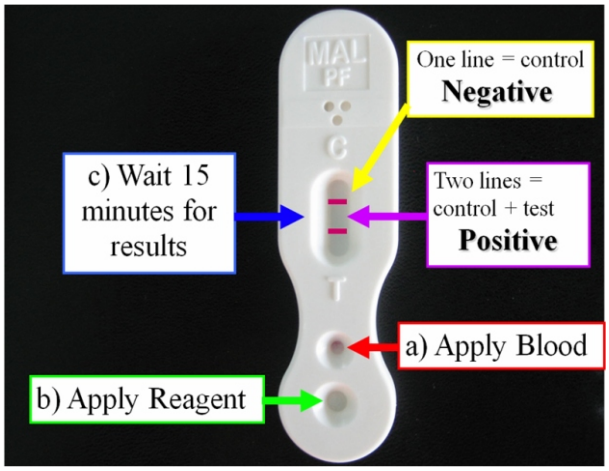
Microscopy, to date, has remained the 'gold-standard' of malaria diagnosis since Laveran made the first microscopic demonstration and identification of the malaria causative agent, *Plasmodium*, in 1880.[14] The technique has had very little change with minor improvements of staining techniques by Romanowsky in the 19th Century. The conventional microscopy diagnosis is by staining (Giemsa, Wright's or Field's stains) thin and thick peripheral blood smears. The technique is widely accepted worldwide due to its simplicity, low cost, high specificity and ability to assess parasite density. Studies continue to demonstrate its benefit in aiding effective management of malaria in endemic countries such as Sub-Saharan Africa particularly in this region of Southern Africa.[45] Though microscopy has high specificity; its sensitivity is unfortunately very low especially at low parasite density. A study in Tanzania among 10 hospitals showed that over a third (39%) of the so diagnosed positive slides were actually false positive.[48] Another similar study in Kenya of 17 outpatient clinics showed that the positive predictive value of 'positive' slides was only 22 %.[42] Indeed, O'Meara et al, have also demonstrated that even among highly trained microscopy personnel there is high inter-expert discrepancy in malaria microscopy parasite readings.[47] To counter-balance these limitations, continued retraining of personnel, such as microscopists, is necessary and in some instances such efforts have demonstrated positive results.[48] In addition, traditional routine microscopy can be reinforced by other concentration techniques such as the Quantitative Buffy Coat (QBC). However, overall, microscopy still poses a lot of challenges including labor intensiveness of the technique, low sensitivity, need of high diagnostic expertise, and requirement of minimum infra-structure (microscope, power/electricity, etc).

Rapid Diagnostic Tests (RDTs)

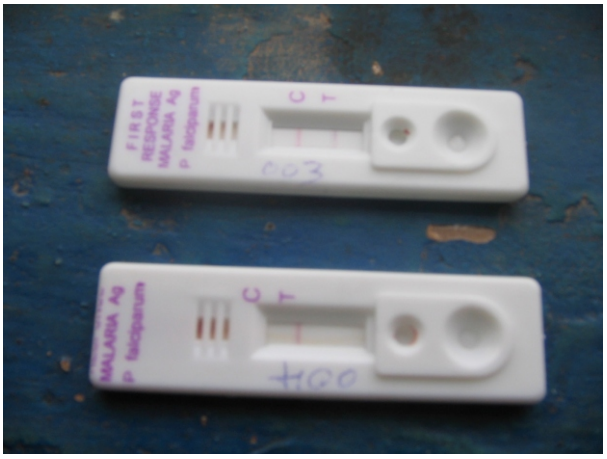
The World Health Organization (WHO) has recently endorsed and ushered in RDTs as the new simple, quick, accurate and cost effective diagnostic tests for determining the presence of malaria parasites and thus providing a suitable alternative to microscopy in the effective and prompt diagnosis of malaria.[43] RDTs currently available in the market are quite a few[49] and include brands such as OptiMAL, Paracheck, ICT, para-sight-F, parascreen, and SD Bioline. Unlike the various microscopy techniques, RDTs do not require laboratory equipment and are all based on the same principle and detect malaria antigen in blood flowing along a membrane containing specific anti-malaria antibodies (see Figure 2). Most of the available RDTs are *P. falciparum* protein specific (either histidine rich protein II -HRP-II or lactase dehydrogenase-LDH) while some RDTs detect *P. falciparum* and other *Plasmodium* proteins such as aldolase or pan-malaria pLDH. Several studies have reported the performance of RDTs to be excellent.[33-34, 50-52] Inarguably, RDTs are enhancing the benefits of parasite-based diagnosis of malaria though not without problems or limitations. RDT limitations currently reported and experienced[14,49] include variation in sensitivity, inability to be used as 'stand alone' diagnostic test, lack of community and Health worker confidence in them as recently documented locally[53] and elsewhere[54] and failure to detect mixed malaria infections.

Figure 2.*Plasmodium falciparum* Histidine Rich Protein II (HRP-II) Based Rapid Malaria Diagnostic Test (RDT)

A. Various parts of an RDT Test Strip



B.An Actual RDT test Result: **Positive** and **Negative** test.



Malaria Serological tests

Serology malaria diagnostic tests utilize the principle of detecting antibodies against asexual blood stage malaria parasites. Among these tests, immunofluorescence antibody testing (IFA) is reportedly the most reliable one.[55] IFA titres of >1:20 are said to be positive, < 1:20 is said to be unconfirmed malaria and > 1:200 titres are classified as recent infections.[56] IFA is simple, sensitive but time consuming and requires an expensive fluorescent microscope together with trained personnel.

Molecular Malaria laboratory diagnostic tests

Molecular malaria techniques such as **PCR** on blood or, more recently, even on saliva samples devised in Zambia by Mharakurwa et al[57], the loop-mediated isothermal amplification (**LAMP**), microarray, mass spectrometry (**MS**), and flow cytometry (**FCM**) assay techniques are all new developments mainly utilized in research settings than during routine patient care.[13] These malaria molecular techniques are, however, offering novel ways and strategies in malaria diagnosis.

CONCLUDING REMARKS: RATIONAL APPROACH TO MALARIA DIAGNOSIS

Current evidence indicates that no single method for the diagnosis of malaria is perfect nor can any one of them be a stand-alone accurate and effective diagnostic criterion (Table 1). Accurate and effective malaria diagnosis should thus involve a rational approach to each patient with suspected malaria employing both symptoms/signs-based and laboratory-based malaria diagnostic methods. The prioritizing of any of the malaria diagnostic methods, at all times, should be influenced by various factors including malaria endemicity, transmission pattern, the urgency of the diagnosis, the experience of the health worker, effectiveness of the health care system, and available budget resources.

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Table1. Summary of Comparative analysis of Sensitivity and Specificity of Various Malaria Diagnosis

Malaria Diagnosis method	Sensitivity	Specificity	References
Clinical (Symptoms & clinical signs-based)	0-100%	0-9%	13,32,33,35
Microscopy	0-40%	66-91%	13,42,48,47,58
Malaria Serological Tests	68.2-95.5%	92.2-96.1%	13,55,56
Rapid Malaria Diagnosis Tests (RDTs)	3-100%	52-99.5%	13,33,34,50,51,52

Note: The cited comparative analysis of sensitivity and specificity of the various diagnostic methods is across the various malaria parasite species

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