Malaria Diagnosis: Current Approaches and Future Prospects

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Abstract- Scaled up efforts by a consortia of organisations in the diagnosis, treatment and prevention of malaria have led to a significant reduction in the overall malaria mortality and morbidity in the past few years. Malaria has, nonetheless, remained one of the world’s most burdensome diseases with the over 214 million cases and 438,000 deaths recorded in 2015 (2.68% of global DALYs). This burden is unevenly domiciled in sub-Saharan Africa where 89% of all cases and 91% of all deaths occurred. These figures however, only represent a fraction of the actual global burden of Malaria as surveillance fails to cover most cases in sub-Saharan Africa where the majority of malaria endemic regions lack facilities for diagnosis, case management and active surveillance. The emergence of drug resistant strains of the Plasmodium species prompted WHO to recommend a confirmatory diagnosis of each case of Malaria before treatment. The workability of this recommendation however, begs to be questioned as the majority of all malaria diagnosis is done via Clinical diagnosis; which lacks precision, is still the major form of diagnosis in many malaria endemic regions, and contributes to the over-diagnosis of malaria and subsequent under-diagnosis of other febrile illnesses. Of higher import is the risk of the emergence of drug resistant species due to the unregulated antimalarial use caused by inaccurate clinical diagnosis. Microscopy, which is the gold standard of malaria diagnosis, and the Rapid Diagnostic Tests (RDT) for malaria antigens have proven to be very useful in the diagnosis of malaria giving high levels of specificity and sensitivity. They however have the downsides of having relatively high limits of detection, invasiveness, being labour intensive and expensive in the light of the low income countries where malaria is endemic. More sophisticated tools such as those that employ nucleic acid techniques (Polymerase Chain Reaction and Gene probes) are not field deployable and are mostly applied for research purposes. This necessitates the need for new diagnostic approaches that are suited to the conditions found in malaria endemic regions. A range of novel diagnostic tools with a do-it-yourself approach, leveraging on previously untapped diagnostic material such as urine are currently being assessed. These novel tools promise great results if successful. This review presents an overview of current diagnostic methods, the prospects in malaria diagnostics and finally makes an effort to recommend what an ideal malaria diagnostic tool should be made up of, all the while focusing on sub-Saharan Africa.

Keywords- Malaria, Diagnosis, sub-Saharan Africa

I. INTRODUCTION

The end of the Millennium Development Goals (MDGs) necessitated the switch to Sustainable Development Goals (SDGs). A great deal of progress was made during the 15 years of MDGs as it relates to malaria. The world saw a 37% and 60% reduction in malaria incidence and mortality with increased coverage of key interventions such as Insecticide Treated Nets, preventive treatment in pregnancy and increased use of rapid diagnostic tests[1], there is however a great deal of work to be done as there are still many issues threatening the new goal of eradicating malaria by 2030.

The WHO in its recent strategy forelimination has identified malaria diagnosis as a major factor in getting to zero come 2030 as prompt and accurate diagnosis is the mainstay for effective disease management and surveillance[2]. Currently, malaria diagnosis rests majorly on the Microscopic detection of parasites and Rapid Diagnostic tests (RDT)[3]. These two tools have proven really effective in times past but may fall short in playing their role towards malaria elimination viz-a-viz their shortcomings.

A. Objectives

This review considered the status of currently used diagnostic methods as well as prospective tools in delivering the information which will help inform policy in a bid to eliminating malaria in sub Saharan Africa where the bulk of the burden lies.

B. Methods

In this review, keywords such as ‘malaria’, ‘diagnosis’, ‘issues’ and ‘prospects’ were put into the google scholar databases to find relevant papers. Papers with a focus outside sub-Saharan Africa or falciparum malaria were excluded from this review.

II. MALARIA LIFE CYCLE & DISTRIBUTION

The malaria life cycle is summarized in Figure 1. It typically begins when an infected female Anopheles mosquito takes a blood meal injecting sporozoites into the human bloodstream. These sporozoites travel to the liver, infecting hepatocytes to initiate the pre-erythrocytic phase. About 2 weeks after, the hepatocytes rupture, releasing merozoites which go on to infect erythrocytes, initiating the erythrocytic phase of the infection which is responsible for the symptoms
observed during malaria infection. This phase is also marked by the breakdown of haemoglobin into an insoluble malaria pigment of diagnostic importance called haemozoin. Merozoites become ring-like trophozoites, which form the basis for malaria diagnosis via microscopy. Subsequently, trophozoites divide repetitively to give schizonts which go on to develop into merozoites. The new merozoites burst out of the erythrocytes and infect other erythrocytes. Some merozoites, however develop into male and female gametocytes which lay dormant until picked up by a mosquito during a blood meal. The gametocytes fuse to form a zygote in the stomach of the insect vector after which the zygote develops into sporozoites which travel to the salivary gland from where they are injected into a human during the next blood meal starting the cycle again[4].

Malaria symptoms include fever, headache, chills and vomiting and in severe cases severe anaemia, respiratory distress in relation to metabolic acidosis, or cerebral malaria[5].

Malaria is currently endemic in most of sub-Saharan Africa (Fig. 1). There were 214 million cases of malaria and 438 000 deaths globally with about 90% of all global cases occurring in the sub-Saharan region[3]. Of particular risk are children and pregnant women who suffer the most from the scourge of malaria.

![Figure 1: Life cycle of malaria (right), P. falciparum incidence in 2015](image)

III. MALARIA DIAGNOSTIC TARGETS

Malaria diagnosis has been predominantly invasive, utilizing whole blood as its primary diagnostic material. Recent efforts, however have been given to the application other non-invasive materials such as saliva and urine as diagnostic materials in malaria diagnosis[7]–[10]. Malaria diagnosis targets whole cells, such as infected Red blood cells (iRBCs) and leukocytes that have ingested parasites or detectable analytes such as parasite antigens, haemozoin crystals and nucleic acids. These targets have effective thus far in malaria diagnosis but not without their individual limitations. The ability and accuracy at which these targets are detected is dependent on the technology used and such factors as reagents, operator proficiency, sample quality and volume. Whole cell targets are especially useful as they give current information on the state of the parasite in the human body as such, they inform treatment and intervention decisions.

Antigenic targets of malaria diagnosis include the *Plasmodium falciparum* histidine rich protein-II (HRPII), lactate dehydrogenase (pLDH) and aldolase. Aldolase is not expressed by *P. falciparum* as such it won’t be considered here. HRPII is a water soluble protein antigen expressed by *P. falciparum* trophozoites and localized in several cell compartments. The parasite releases a substantial amount of the protein into its host’s blood stream making it detectable in blood stream[11], [12], cerebrospinal fluid and urine[13]. HRPII however persists in the blood stream for up to two weeks[14] after parasite must have been cleared greatly limiting its diagnostic efficacy as a diagnostic target. This persistence has the implication of contributing to the over diagnosis and subsequent over treatment of malaria[15], [16]. pLDH is the last enzyme in the glycolytic pathway essential for ATP generation. As opposed to HRPII, the amount of pLDH in the blood stream reduces as parasites clear from the blood stream as such it can be used to monitor treatment outcome[17].

The most suitable diagnostic targets, however would be one that accurately tells the current condition of infection viz-a-viz parasite biomass and species identification

IV. CURRENT METHODS IN MALARIA DIAGNOSIS

A. Clinical Diagnosis

Clinical diagnosis, also known as presumptive diagnosis, is the most practiced method of diagnosing malaria as it is very cheap and rapid[18]. It is based on the signs and symptoms presented by the patient. These signs and symptoms are often non-specific and include fever, headache, dizziness, cold, nausea, pruritus and anorexia [19]. Clinical diagnosis however possess many problems due to the overlap of malaria symptoms with those of other common infections found in endemic regions such as bacterial infections. This overlap of symptoms with other diseases greatly reduces the specificity of clinical diagnosis ergo contributing to the over diagnosis and over treatment of malaria as shown in various studies with one reporting as high as 83% over diagnosis and over treatment of malaria in children in the age group of 1-5 years of age[20], [21].

Clinical diagnosis is most useful in areas where there is no laboratory support which is often the case in many areas in
sub-Saharan Africa, it is especially useful, and has been recommended by the integrated child management initiative (IMCI), in treating children, 5 years and below, in these areas as malaria infections can quickly become fatal for this age group[19], [21]–[23].

The sensitivity of clinical diagnosis is determined by factors such as endemicity, season and age group with accuracy of clinical diagnosis increasing with prevalence and endemicity[19], [21].

The combination of clinical diagnosis with other parasite based methods greatly increases the accuracy of malaria diagnosis.

B. Microscopy

Microscopic examination of Giemsa-stained blood smears remains gold standard for the diagnosis of malaria. Diagnosis is done by the examination of Giemsa stained and/or thick blood smear. When performed under optimal conditions by a competent microscopist, microscopy can detect as low as 5 iRBCs, however its sensitivity can go as low as 200iRBCs when conditions are not optimal [24], [25].

Microscopic examination of blood smears allows for the diagnosis of infection in symptomatic and asymptomatic persons depending on the skill of the microscopist, parasitemia and the number of high powered fields observed[26], [27]. It also has the advantage being both qualitative (species, stage identification) and quantitative hence it’s preferred use in the monitoring of patient response to treatment. Microscopy, however falls short in that it requires expertise for accurate results, maintenance costs, need for constant supply of electricity and others as summarized in table 1. Sampling preparation also greatly affects the sensitivity of this test as the quality of the film, duration of staining and quality of stain affect the ability to visualize the different stages of the parasite [18].

Recent advances in technology have given rise to new ways of manipulating blood in a bid to concentrate cells before analyses. The exposure of blood samples to a magnetic fields helps concentrate iRBC as a result of the presence of haemoglobin[28], [29]. This increases the sensitivity of the test by as much as 100 fold. Other methods to increase sensitivity include the centrifugation of heparinized blood to concentrate the different stages of parasite growth before making a film[30] however, its application in Africa is not well documented.

The advent of power packs and solar batteries have made microscopy more field deployable hence increasing its efficacy in resource poor areas. Nonetheless, there is still a dearth of experienced microscopists in the sub-Saharan African region making microscopy a less effective method in these parts [31].

C. Immunochromatographic Rapid Diagnostic Tests

The Rapid Diagnostic Test (RDT) is a very effective tool in malaria diagnosis and forms the mainstay of diagnosis in many resource poor areas where there is no access to a laboratory. It is especially useful as it requires no electricity, infrastructure, minimal sample preparation, technical expertise and interpretation of results is relatively easy.

The RDT detects malaria antigens in 5-15 µL of blood using monoclonal antibodies, impregnated on a test strip, specific for the targeted antigens, in an immunochromatographic assay. Test results are interpreted by the absence or presence of a coloured line on the strip and can be obtained in 5-20mins. RDTs detects three plasmodial antigens; P.falciparum histidine rich protein II (PfHRPII), Plasmodium lactate dehydrogenase (pLDH) and aldolase [17], [32]. Most RDT products incorporate two of these three antigens in a bid to distinguish falciparum from non-falciparum infections.

HRPII antigen is specific for P.falciparum and is a major constituent of RDTs in sub-Saharan Africa, it however has the shortcoming of persisting for up to 2 weeks after parasite must have cleared from the blood stream as such it is not effective in monitoring treatment[33], [34]. There have been reports of false negatives from HRPIIRDT kits as a result of a mutated or deleted HRPI gene [35]. It is recommended that regions with more than 10% prevalence of HRPII deletion should seek alternatives such as microscopy for the diagnosis of malaria.

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D. Nucleic Acid Techniques

Tests that target nucleic acids have proven to be one of the most specific and sensitive methods with the ability to detect parasitemia of 0.005 iRBC/µL. They especially come in handy in detecting sub-microscopic infections which greatly threaten the elimination strategies that abound. Nucleic acid techniques are not yet field deployable as they are expensive, require expert personnel and specialized equipment as such, their use is limited to clinical research in population diversity studies[40] and monitoring of drug resistance[41].

Nucleic acid techniques target gene sequences such as the 18S ribosomal RNA gene sequences[42], P.falciparum stevor multigene family[43], mitochondrial DNA, and telomere-associated repetitive element-2[44]. Nucleic acid techniques include the Polymerase Chain Reaction and its various methods (real time PCR. Nested PCR) which use specific primers to make many copies of a small amount of template DNA, followed by post-PCR methods such as gel electrophoresis. The PCR has the advantages of being able to process many samples at once.

Another nucleic acid technique is the Loop-mediated isothermal amplification (LAMP) technique. This technique has the potential to make nucleic acid strategies field deployable, as it requires lesser instrumentation than PCR and is easy to use. LAMP in contrast to PCR is an isothermal technique requiring a constant temperature for amplification. The amount of DNA produced in LAMP rivals that of PCR as there is no need for a constant temperature for amplification. Amplification is detected by turbidimetry due to an increasing quantity of magnesium pyrophosphate and as such can be followed in real-time[45]. Some studies have reported the technique’s ability to detect SMI and sensitivities that surpass that of RDT and rival that found in PCR[45]-[47]. LAMP is easy, sensitive and cheaper than PCR, it however has the downside of the need for cold storage of reagents [18].

V. PROSPECTS IN MALARIA DIAGNOSIS

Recent efforts in malaria diagnosis have been targeted towards utilizing other non-invasive diagnostic materials in a bid to making testing procedure less painful for most. Saliva[7], [8], [48] and urine[9], [13] have been assessed as diagnostic material with promising results reported thus far.

A cheap urine based RDT with the ability to deliver results in 25 minutes, is currently undergoing evaluation and has shown positive reports with a reported limit of detection of 125 parasites/µL[13] which is well within the range recommended by the WHO [32]. If successful, this new tool could finally usher in the era of non-invasive diagnosis of malaria. Some studies have also shown the detection of parasite DNA in saliva and urine howbeit in smaller quantities than those found in the blood[9]. They have however been shown to be suitable alternatives to blood where blood samples are not available [49]-[51].

<table>
<thead>
<tr>
<th>TABLE 1: Summary of currently used diagnostic techniques</th>
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<tr>
<td><strong>Adantages</strong></td>
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<td>:-----------------:</td>
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<tr>
<td><strong>Clinical Diagnosis</strong></td>
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<tr>
<td>Useful in resource poor areas</td>
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<tr>
<td>Particularly useful in preventing infant mortality</td>
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<tr>
<td>in resource poor regions</td>
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<tr>
<td><strong>Microscopy</strong></td>
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<tr>
<td>Low Tech, simple and inexpensive</td>
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<tr>
<td>Field Deployable</td>
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<tr>
<td>Identifies parasite itself</td>
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<tr>
<td>Useful in monitoring treatment outcome</td>
</tr>
<tr>
<td><strong>RDT</strong></td>
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<tr>
<td>Field deployable</td>
</tr>
<tr>
<td>Easy to use</td>
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<tr>
<td>Requires no expertise</td>
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<tr>
<td>Can detect <em>P.falciparum</em></td>
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<tr>
<td>Zero to minimal sample preparation required.</td>
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<tr>
<td><strong>Nucleic Acid Techniques</strong></td>
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<tr>
<td>Highly sensitive</td>
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<tr>
<td>Ability to detect SMI</td>
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<tr>
<td>Can be used to investigate population diversity and distinguish between new and recrudescent infections</td>
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<td>High throughput</td>
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Other strategies seek to exploit the electromagnetic and physical properties of haemozoin crystals formed during the malaria life cycle [28], [52], [53]. Haemozoin based detection strategies have the added advantage of giving adequate diagnosis during sequestration of parasites. They may however not be field deployable as some strategies do not detect haemozoin in early ring stage parasites [54] while most other techniques require specialized equipment and personnel.

Another emerging diagnostic tool are the aptamers. Aptamers are single-stranded oligonucleotides made in-vitro which possess a specific three-dimensional structure depending on its sequence [55]. Aptamers interact specifically with their targets by binding to them and altering their activity. Aptamers targeting pLDH have been designed and tested with promising results[56], [57]. It is expected that this technology will be perfected and field deployed in the nearest future.

VI. CONCLUSION

The conventional microscopic examination of blood films remain the gold standard as it is cheap and offers sensitivity unrivalled by any other field deployable method. RDTs are convenient but could lead to over diagnosis and over treatment. Nucleic acid techniques offer the best sensitivities, they are however expensive, out of reach to most and are limited to clinical research. Getting to zero would require
diagnostic tools that are non-invasively highly sensitive, robust and suited to the conditions found in the endemic regions.

REFERENCES


