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A comparative laboratory diagnosis of malaria: microscopy versus rapid diagnostic test kits

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ABSTRACT

Objective: To compare the two methods of rapid diagnostic tests (RDTs) and microscopy in the diagnosis of malaria. **Methods:** RDTs and microscopy were carried out to diagnose malaria. Percentage malaria parasitaemia was calculated on thin films and all non-acute cases of plasmodiasis with less than 0.001% malaria parasitaemia were regarded as negative. Results were simply presented as percentage positive of the total number of patients under study. The results of RDTs were compared to those of microscopy while those of RDTs based on antigen were compared to those of RDTs based on antibody. Patients' follow-up was made for all cases. **Results:** All the 200 patients under present study tested positive to RDTs based on malaria antibodies (serum) method (100%). 128 out of 200 tested positive to RDTs based on malaria antigen (whole blood) method (64%), while 118 out of 200 patients under present study tested positive to visual microscopy of Lieshman and diluted Giemsa (59%). All patients that tested positive to microscopy also tested positive to RDTs based on antigen. All patients on the second day of follow-up were non-febrile and had antimalaria drugs. **Conclusions:** We conclude based on the present study that the RDTs based on malaria antigen (whole blood) method is as specific as the traditional microscopy and even appears more sensitive than microscopy. The RDTs based on antibody (serum) method is unspecific thus it should not be encouraged. It is most likely that Africa being an endemic region, formation of certain levels of malaria antibody may not be uncommon. The present study also supports the opinion that a good number of febrile cases is not due to malaria. We support WHO's report on cost effectiveness of RDTs but, recommend that only the antigen based method should possibly, be adopted in Africa and other malaria endemic regions of the world.

1. Introduction

Malaria is one of the highest killer diseases affecting most tropical countries especially Africa. It affects over 500 million people world wide and over one million children die annually from malaria[1]. Of all the human malaria parasites *Plasmodium falciparum* (*P. falciparum*) is the most pathogenic and is frequently fatal if untreated in time[2]. In India, according to Nandwani[2] a total of 1.82 million cases of malaria and 0.89 million cases of *P. falciparum* cases with 902 death were reported in the year 2002.

Traditional practice for outpatients has been to treat presumptively for malaria based on a history of fever but, a significant proportion of those treated may not have parasites (over 50% in many settings) and hence waste a considerable amount of drugs[3]. This old clinical based practice is still relevant today especially, in infants where time spent on getting a confirmatory laboratory diagnosis could lead to increased fatality.

Widespread prescription of chloroquine to patients not having malaria has been tolerated, partly because chloroquine is so cheap. However, artemisinin-based combination therapy (ACT) costs at least 10 times more per treatment. Moreover, overdiagnosis of malaria implies underdiagnosis and inappropriate treatment of non-malarial febrile illness while a high proportion of such illnesses are self-limiting viral diseases, and a significant minority, such as acute respiratory infections or bacterial meningitis, are bacterial diseases and potentially fatal[3].

WHO currently makes the tentative recommendation that parasite-based diagnosis should be used in all cases of suspected malaria with the possible exception of children

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in high-prevalence areas and certain other situations[4,5]. For this recommendation to be adhered to obviously, rapid and accurate laboratory finding or demonstration of malaria parasite should be established.

The traditional method of microscopic identification of parasite however, is not only daunting in poor power setting, but also time consuming and requiring a lot of expertise/training. Thus microscopy in Africa is generally, limited to larger clinics/tertiary centers. This conventional staining of peripheral blood smears/microscopy however still remains the gold standard in laboratory diagnosis of malaria[2].

Rapid diagnostic tests (RDTs) for malaria could be considered for most patients in endemic regions, especially in poor power settings where there is shortage of qualified manpower in Africa. However, there is very little evidence, especially from malaria endemic areas to guide decision-makers on the sensitivity and specificity of these RDTs.

RDTs are commercially available in kit forms with all necessary reagents and the ease of performance of the procedures, does not require extensive training or equipments to perform or to interpret the results. Results are read in 12-15 min[6].

RDTs mainly come in two forms. One is antigen based and normally requires the use of haemolyzed red blood cells while the other is antibody based and normally requires the use of extracted serum. Generally speaking, antibodies are better expressed in serum otherwise plasma could also stand in place of serum for antibody based method.

The principles of tests stem from detection of malaria parasites' protein *i.e.* histidine. Where antibody method is used, it means detection of the presence of antibodies against histidine in the human serum and where whole blood is used, it implies detection of malaria parasites' histidine on the red blood cells[6].

Therefore, the study was aimed to compare the two methods of microscopy and RDTs in the diagnosis of malaria.

2. Materials and methods

Materials consisted of ethylene diamine tetraacetic acid (EDTA) blood bottles, plain Khan tubes, 5 mL syringes, Lieshman and Giemsa stains, microscopic slides, light microscope with good 40× and 100× objectives, RDT kits from SD-Diagnostics USA and KS LAB-China.

Blood samples were collected into EDTA and plain Khan tubes from a total of 200 patients who presented with fever for 1-3 days and were clinically diagnosed of malaria fever.

Thick and thin films were made in triplicates from EDTA samples within 10 min of collections while sera were harvested from the plain tubes as soon as clots were fully formed.

Thick films were stained by Giemsa's, and Field's methods while the thin films were stained by Lieshman's and diluted Giemsa's methods.

RDT based on antigens was carried out on aliquots of haemolysed whole blood in duplicates. Sera were tested in duplicates to detect malaria parasites antibody based on RDTs-antibody detection method.

Percentage malaria parasitaemia was calculated on thin films and all non-acute cases of plasmodiasis with less than 10% malaria parasitaemia were regarded as negative.

Percentage malaria parasitaemia was calculated as well as average percentage malaria parasitaemia suppression. These calculations were done using the below formula.

$$\%\ \text{Malaria parasitaemia} = \frac{\text{No of MP}}{\text{Total No of WBC}} \times 100$$

Where, WBC=white blood cells, No=number and MP=malaria parasites (WBC in the case of thick film or RBC in the case of thin films).

Twenty microscopic fields with an average of 50 WBC per field were counted to give a total of 1 000 WBC as counted No of WBC. Where thin films were examined, RBC replaced WBC in the above formula for calculating percentage parasitaemia[1].

A day later, follow-up was made for all patients from day 2 to 4. All patients received antimalarial along with antibiotics/antibacterial drugs.

All blood films with more than 0.001% (≤50/μL) positive malaria parasitaemia and with visual malaria parasites were simply presented as positive while those of less than 0.001% and without visual malaria parasites were simply taken as negative. RDTs for both antigen and antibody based were also simply reported as positive or negative. Percentage fraction of total number of patients (200) was reported for all methods.

3. Results

The results showed that it could possibly be appreciated that the serum method appeared unreliable as a specific method of malaria diagnosis. Since microscopy was adopted as the gold standard, all the 72 that came out negative with antigen method still had negative results with microscopy. While 10 extra negative cases with microscopy were positive with antigen method (Table 1). The antigen method therefore could be said to be more reliable than antibody method and equally as specific as the gold standard *i.e.* microscopy.

From Table 2, most species of *Plasmodium* microscopically demonstrated were trophozoites of falciparum while very few cases had gametocytes of the same *P. falciparum*. Our RDTs were limited to histidine rich proteins 2 (HRP-2) which is a feature of *P. falciparum* and 40-50 min was spent in microscopic search after which a result was declared. There were occasional public electric power outages during microscopy but, time taken to run the generator was not built so the average time could possibly be up to or over an hour. It took longer time to process thin films than thick film whereas there was no significant time taken to bring the strips or cassettes out of the sachets of RDTs packets. The average migration time was 9-17 min for antigen method whereas that of serum was very rapid and completed in 3-7 min. Reason for slower migration for that of antigens based

may be a factor of viscosity or fragmented red blood cells sludge in the bottom of containers.

Table 1
A summary of positive/negative figures.

Items	Whole blood (antigens)	Serum (antibody)	Whole blood films
Negative	72	NIL	82
Positive	128	200	118
Total	200	200	200
Percentage positive	64%	100%	59%

Table 2
A summary of average time taken per method/species.

Items	Stain-time (min)	Com-time (min)	Species
Whole blood (antigens)	NIL	13±4	HRP-Pf
Serum (antibody)	NIL	5±2	HRP-Pf
Whole blood thick films	3±1	40±10	Tropho & Gameto Pf
Whole blood thin films	12±4	40±10	Tropho & Gameto Pf

HRF: Histidine rich protein; Pf: *P. falciparum*; Tropho: Trophozoite; Gameto: Gametocyte.

4. Discussion

There are four principal methods for diagnosing malaria. These are symptomatic, microscopy, antigen test and molecular methods. Symptomatic diagnosis is the most common, and people in poorer countries often use symptoms alone to diagnose malaria. In other areas, too, symptomatic diagnosis is often the initial one, followed by one of the other methods. However, it should be noted that many other diseases present symptoms very similar to malaria, and diagnosis by symptoms alone can be misleading and even harmful. Treating for malaria where other treatment is called for leaves the actual disease uncured and the patient in critical condition. It is therefore imperative to follow up symptomatic diagnosis with one of the other more accurate methods.

Onset of long periodic fevers, chills and bodily pain are often taken together to be symptoms of malaria. However, this diagnosis is often wrong; so at times is parasitemia, which means the concentration of parasites in the blood; both can be caused by other sorts of infections. It has been shown that retinopathy, the study of changes occurring in the retina of the eye, can give good indication of malaria, because the color and other aspects of retinas were changed as a result of particular diseases. A percentage parasitaemia need therefore be adopted to correlate with clinical presentation.

Microscopic examination of blood, ever since the singular discovery of Laveran, the French scientist who first identified the plasmodium parasite, is the most reliable method of diagnosing malaria. Therefore, a specimen of blood is observed under the microscope for presence of the malaria parasite. Although, other bodily fluids like saliva or urine can also be used as less invasive methods, blood is preferred for higher concentration of the parasite.

WHO currently makes the tentative recommendation that parasite-based diagnosis should be used in all cases of suspected malaria with the possible exception of children in high-prevalence areas and certain other situations[4,5]. For this recommendation to be adhered to obviously, rapid and accurate laboratory finding or demonstration of malaria parasite should be established. The introduction of high-cost antimalarial drugs such as ACT is encouraging in malaria-endemic countries in sub-Saharan Africa to reassess diagnostic practices[3]. This drive to have rapid and accurate method of malaria diagnosis led Nandwani *et al*[2] to carry out a comparative malaria detection between polymerase chain (PCR) reaction and microscopy on 100 Indian patients. They found the PCR method to be 96.8% sensitive but took about 10-11 h to complete whereas microscopy took an average of 40-45 min. PCR detects the presence of malaria parasites on/in the red blood cells. PCR requires electric power which costs a fortune and cannot easily be considered for use in Africa. Also it is very time consuming and does not meet our speed desire.

Immunochromatographic method to detect the presence of malaria parasite appears to be the most rapid and requires minimum or no training at all. Immunochromatographic method relies on the migration of liquid across the surface of a nitrocellulose membrane[6]. The test is based on the capture of parasite antigen from the peripheral blood using monoclonal antibodies prepared against malaria antigen target and conjugated to either a liposome containing selenium dye or gold particles in a mobile phase. Or reversed, where in place of monoclonal antibody to capture the antigen, antigen is incorporated into the cellulose to capture the antibody in the serum or plasma. Our present study confirms that the immunochromatographic method represented as RDTs is the most rapid with the antibody method being most rapid.

Microscopy is the most widely tool used to diagnose malaria at peripheral levels. In capable hands it is very sensitive for parasitaemia ≤50/μL (0.001%)[2] and it can give important information to the clinician like species, parasites stages and parasite density. However, good quality of microscopy is difficult to implement and maintain. It is labor intensive and requires highly skilled personnel and regular quality control. The use of malaria RDTs is recommended by WHO when reliable microscopy is not available. In non-endemic settings, where microscopic expertise is lacking due to low incidence, malaria RDTs are of value for the diagnosis of malaria and they provide information about the involvement of *P. falciparum*. In a recent external quality control session, 72.7% of 183 Belgian laboratories offering malaria diagnosis declared to use RDTs as a tool for diagnosis, and their use is recommended if performed in conjunction with microscopy[7]. Maltha *et al*[7] also showed that *P. falciparum*, *Plasmodium vivax* and *Plasmodium malariae* showed 94.6%, 92.9% and 94.7% degree of sensitivity using RDTs in malaria parasites concentration of ≥1000/μL, respectively, but, they showed percentages lower than an average of 58% sensitivity in malaria concentration of ≤100/μL. It should naturally, be expected that their sensitivity will drop to almost zero at concentration of 0.001% (≤50/μL) where also

microscopy should be negative.

Malaria antigens currently targeted by RDTs are HRP-2, parasite lactate dehydrogenase (pLDH) and plasmodium aldolase (PL-aldo). Moody[6] demonstrated that *Plasmodium* species secret these proteins thus the sensitivity and specificity of RDTs are measured based on them. *P. falciparum* has been shown to secret lots of HRP-2 more than HRP-1 and HRP-3 whereas pLDH and PL-aldo are found in other species of *Plasmodium*.

Membranes of erythrocytes infected with the human malaria parasite *P. falciparum* develop protrusions called knobs. These structures are essential for the survival of the parasite in the host, and their induction requires the synthesis of the knob protein by the parasite[8-14]. These knobs are rich in histidine. Histidine existing in man as an essential amino acid, has a positively charged imidazole functional group. The imidazole makes it a common participant in enzyme catalyzed reactions. The unprotonated imidazole is nucleophilic and can serve as a general base, while the protonated form can serve as a general acid. The residue can also serve as a role in stabilizing the folded structures of proteins. The histidine found on malaria parasites is an isomer and RDTs are sensitive only to that of malaria (HRP-2). This type is however found only in *P. falciparum* while the pLDH is found in the other species. But, most RDTs will not detect the presence of malaria parasites in mixed species infection[15]. Our RDTs method was more sensitive to HRP-2 since *P. falciparum* is not only the most fatal but also the most commonly found in this part of the world, then it is justifiable. The microscopy, the gold standard also falls in line with this view. It must however be noted that since all patients under current study had antimalaria and were all relieved of their ill-health, the possibility of other species of *Plasmodium* may exist.

Our work also shows clearly that the antigen based method has a better correlation with both the gold standard *i.e.* microscopy and the clinical settings. The antibody based method as anticipated showed good level of sensitivity but, very unspecific. Nigeria is a malaria endemic area, so antibodies against HRP-2 may be a common finding. In our present work, it was 100% and if this is extrapolated to our larger society, it means that virtually everyone that is febrile will test positive to the antibody method.

We conclude that the RDT for diagnosis of malaria is as reliable as microscopy but, only the antigen based method is suitable in Nigeria and perhaps other parts of the tropics where malaria is endemic. We recommend that only the antigen based method kits be imported and, or used in Nigeria and perhaps other parts of the tropics with malaria endemicity.

Conflict of interest statement

We declare that we have no conflict of interest.

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