Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage:www.elsevier.com/locate/apjtb

Document heading

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

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ARTICLE INFO

Article history:
Received 15 October 2011
Received in revised form 3 November 2011
Accepted 27 November 2011
Available online 28 April 2012

Keywords:
Rapid diagnostic tests
Microscopy
Malaria diagnosis
Malaria
Parasitaemia
Plasmodiasis
Antigen
Antibody
Antimalaria
Serum
Whole blood

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