A comparative study of blood smear, QBC and antigen based rapid diagnostic test for diagnosis of malaria

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Abstract

Background & Objectives: Rapid diagnosis is prerequisite for effective treatment and reducing mortality and morbidity of malaria. Microscopy has been the Gold standard for malaria diagnosis for decades. We made an attempt to compare blood smear, Quantitative Buffy Coat (QBC) and rapid antigen detection methods for the rapid diagnosis of malaria.

Methods & Materials: A retrospective study was conducted for 6 months in G.G. Hospital, Jamnagar. A total number of 90 hospitalized clinically suspected malarial cases were collected and confirmed by conventional blood smear, QBC and antigen based Rapid Diagnostic Test (RDT). Blood smears were prepared and stained with Leishman’s stain. QBC and Rapid Diagnostic tests were done using commercially available kits. Patients were followed-up for signs of clinical recovery.

Results: Malaria was diagnosed in 50, 54 and 59 patients by Leishman staining technique, QBC method and Rapid antigen detection test respectively. Sensitivity, specificity, positive and negative predictive values of Antigen detection test were 94%, 70% 79% & 90%, respectively while, those of QBC were 84%, 77%, 82% & 79%, respectively. The sensitivity of rapid diagnostic test is high with grade 3 & 4 parasitemia.

Conclusion: In our study, rapid diagnostic tests were found more accurate than PBS & QBC. Microscopy is cost effective but microscopy and QBC requires technical expertise to interpret the results. In places where facilities are not available, rapid, simple and easy to interpret antigen detection test can be used, especially in endemic areas.

Keywords: peripheral blood smear, Quantitative Buffy Coat, Rapid Diagnostic test

1. Introduction

Malaria is seen in all countries extending from 40°S and 60°N of equator covering a large portion of tropical and subtropical region. Malaria is a public health problem in more than 90 countries. According to the latest estimates of WHO, there were about 219 million cases of malaria in 2010 (with an uncertainty range of 154 million to 289 million) and an estimated 6,60,000 deaths (with an uncertainty range of 490 000 to 836 000).

Being associated with most serious complications, diagnosis of malaria constitutes a medical emergency. Since prompt treatment can grossly reduce mortality and morbidity associated with malaria, specific of rapid diagnosis of this disease becomes imperative and main emphasis of current WHO malaria control strategy.
At peripheral level of health centers, treatment is generally given following a diagnosis based on clinical symptoms. Chloroquine and other low-cost drugs were highly effective and cost-effective but the emergence of chloroquine resistant malaria has made it urgent to ensure that treatment is based on rapid and reliable diagnosis of disease.

However, over the years many new tests have been developed in an attempt to improve the diagnosis of malaria, but conventional method by smear microscopy remains the gold standard against which all other tests have been evaluated. In recent years, numerous quick and new techniques for malaria diagnosis have been developed, one such being the QBC technique. They require technical expertise and availability of a good quality microscope. Microscopy is difficult to maintain in remote and poorly resourced areas. So it requires the availability of a rapid, sensitive, and specific test at an affordable cost.

RDTs offer the risk to extend accurate parasite-based malaria diagnosis to people in remote areas for the first time. Results of rapid diagnostic tests are rapidly available, less liable to the theoretical risk of being falsely negative due to parasite sequestration, and visible to both prescriber and patient, and they may result in greater respect for test results. They are commercially available kits which include all necessary reagents and do not require extensive training or equipment to perform or interpret their results.  

Keeping in mind the seriousness of the condition and the current availability of diagnostic facilities across India, we decided to conduct a comparative study of the commonly employed diagnostic techniques in diagnosis of malaria, i.e., peripheral blood smear, QBC and antigen detection test.

2. Materials and Methods

A retrospective study was conducted during the period of 6 month in G. G. Hospital, Jamnagar, Gujarat. A total of 90 cases were enrolled in the present study from the different wards of medicine with clinical suspicion of malaria presenting with pyrexia with chills and rigor or atypical presentations were taken for the study. Detailed history and clinical examinations were carried out according to proforma. Blood samples were collected in EDTA bulb from suspected cases and already diagnosed cases of malaria, either by outside (private labs) or by inside (Pathology department). Diagnosis of malaria was suspected by clinical features and confirmed by Peripheral smear examination, QBC and Rapid diagnostic test (Malaria Antigen dipstick).

2.1 Peripheral smear preparation

Thick smear and thin smear were prepared either from finger prick or from EDTA bulb. Both of them were dried and then stained with Leishman’s stain. For thick smear, gentle heat was applied to prevent ‘washing out’ of smears. After staining, the smears were examined under 100X. At least 100-200 fields, each containing 20 WBCs were examined before smear was reported as negative for malaria. The red blood cells in the tail end of the smear were examined for the species identification and stages of the parasites.

2.2 Quantitative Buffy Coat (QBC)

In the QBC technique, the capillary tube was filled with 55 μl to 65 μl of blood and was centrifuged at rate of 12000g for five minutes. The principle of QBC technique is based on the fact that on centrifugation at a high speed, the whole blood separates into plasma, buffy coat and packed red cell layer. Blood cells in the buffy coat layer separated according to their densities. As the parasites within erythrocytes mature, they reduce the buoyant density of infected erythrocyte. These two properties are exploited in QBC technique for malaria diagnosis. Less dense Red cells containing Plasmodia are concentrated just below the leukocytes, at the top of the erythrocyte column. Due to acridine orange, the signet ring forms appeared as distinct apple green dots inside the faint RBC, gametocytes of P. falciparum appeared as yellow sickle-shaped bodies. Schizonts of P. vivax were recognized by the presence of malaria pigment which appeared dark brown colour with oil immersion objective attached to light microscope.

2.3 Rapid Diagnostic Tests (RDT):

Antigen detection tests are most recently developed of the immunochromatographic rapid malaria strip tests. Antigen detection tests detecting parasitic antigens like histidine-rich protein-2 (HRP 2), plasmodium lactate dehydrogenase (pLDH) and pan-specific aldolase. Major advantage with the pLDH test is its specificity. It not only helps to detect the presence of plasmodium species but also differentiate the P. falciparum and P. vivax. The test was done using anticoagulated blood. Commercially available antigen detection kit(Malarigen Pf/Pv) was used. In this kit, the detection system for P. falciparum malaria is based on detection of P. falciparum specific HRP2 and P. vivax specific pLDH. The kits were all from
the same batch and were used before the expiry date and performed according to the instruction manual by the manufacturer. The test was done according to manufacturer’s instructions. Interpretation of the test result was done as below:
1. When one control band and two test bands appeared the test was considered to be positive for *P. falciparum*.
2. When one control band and one test band appeared the test was considered positive for *P. vivax*.
3. When only control band appeared at the top of the test strip without test band the test was considered negative.

2.4 Statistical analysis

Samples were classified as true-positive (TP), true-negative (TN), false-positive (FP) or false-negative (FN) by comparison with a reference standard. Sensitivity (TP/TP + FN) and specificity (TN/TN + FP), as well as positive (TP/TP + FP) and negative (TN/TN + FN) predictive values, for the test were then calculated.

3. Results

During the study period, 90 cases tested for smear examination, 56% (50 out of 90) of the cases were positive by thick blood smear examination, whereas thin blood smear examination showed a positivity of 51% (41 out of 90).

Table-1: comparison of species specificity of malarial parasite by peripheral smear & by rapid diagnostic test

<table>
<thead>
<tr>
<th>Methods</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>P. falciparum</em></td>
<td><em>P. vivax</em></td>
<td>Mixed</td>
</tr>
<tr>
<td>Peripheral smear</td>
<td>33</td>
<td>16</td>
<td>01</td>
</tr>
<tr>
<td>QBC</td>
<td>43</td>
<td>7</td>
<td>01</td>
</tr>
<tr>
<td>Rapid diagnostic test</td>
<td>39</td>
<td>17</td>
<td>03</td>
</tr>
</tbody>
</table>

Above table shows that, total 56% (50 out of 90) cases were positive by peripheral smear examination, of which 36.7% (33 out of 90) were positive for *P. falciparum*, 17.8% (16 out of 90) cases were positive for *P. vivax* and 1.11% were positive for mixed cases. QBC examination was positive for 57% (51 out of 90) cases of which 47.8% (43 out of 90) were positive for *P. falciparum*, 7.8% (7 out of 90) were positive for *P. vivax* and 1.11% (1 out of 90) were mixed cases while, 66% (59 out of 90) of the cases were positive by RDT of which 43.3% cases were positive for *P. falciparum* while 22.2% were positive for non-*falciparum* species (Table 1).

The QBC method allowed an additional 4 cases and Rapid antigen detection test method allowed an additional 9 cases. The majority of variations occurred in smear negative cases.

Table-2: comparative study of peripheral smear and rapid diagnostic test (pLDH):

<table>
<thead>
<tr>
<th>Rapid antigen detection test</th>
<th>Microscopy</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Positive</td>
<td>47 (TP)</td>
<td>12 (FP)</td>
</tr>
<tr>
<td>Negative</td>
<td>03 (FN)</td>
<td>28 (TN)</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>40</td>
</tr>
</tbody>
</table>

Above table shows that by taking thick smear as gold standard, sensitivity, specificity, positive and negative predictive values of Rapid Diagnostic Test were 94%, 70%, 79% & 90%, respectively (Table 2).

Table-3: comparative study of peripheral smear and rapid diagnostic test (pLDH):

<table>
<thead>
<tr>
<th>QBC</th>
<th>Microscopy</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Positive</td>
<td>42 (TP)</td>
<td>09 (FP)</td>
</tr>
<tr>
<td>Negative</td>
<td>08 (FN)</td>
<td>31 (TN)</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>40</td>
</tr>
</tbody>
</table>

Above table shows that by taking thick smear as gold standard, sensitivity, specificity, positive and negative predictive values of QBC were 84%, 77%, 82% & 79%, respectively (Table 3).
Table-4: parasite level detected by blood smear and rapid diagnostic test

<table>
<thead>
<tr>
<th>Grade of parasitemia</th>
<th>RDT</th>
<th>Microscopy positive</th>
<th>Sensitivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>11</td>
<td>2</td>
<td>13</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>1</td>
<td>16</td>
</tr>
<tr>
<td>3</td>
<td>11</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>0</td>
<td>10</td>
</tr>
</tbody>
</table>

Above table shows that sensitivity of RDT for *P. vivax* and *P. falciparum* are 87.50% and 96.97% respectively. Moreover, out of the 90 patients studied, RDT is more sensitive to grade 3 & 4 parasitemia and less sensitive to grade 1 parasitemia (Table 4).

4. Discussion

Due to the serious nature of *P. falciparum* infections, prompt and accurate diagnosis of malaria is essential for effective management to reduce the morbidity and mortality due to the disease. The majority of malaria is found in countries where cost-effectiveness is an important factor and ease of performance and training is a major consideration.

Leishman's or Giemsa stained thick smears are considered to be the 'Gold standard' in diagnosis. However, this is laborious and time consuming and therefore delays diagnosis (60 minutes) [5-7] and its result is depends on the quality of microscope, staining, technique with which blood film is prepared and also the concentration and motivation of microscopist. Leishman stained thick blood film detects malarial parasite when there are 5-20 parasites/μl and thin blood film detects malarial parasite only when there are 50 parasites/μl of blood [8]. The advantages are that its low cost and a permanent record of the smear can be kept. Another advantage is that species identification is done without much difficulty in most of the cases.

Newer techniques like QBC and Antigen detection assays are rapid. QBC is expensive, and requires expertise as there are chances of leaking and breaking of blood filled QBC tubes in the centrifuge. As compared to the blood smear examination, the RDTs can be performed easily in 5 to 30 minutes by the field workers, untrained personnel, travelers, or others with a minimum training of 3 to 24 hours [9, 10].

In this study, rapid diagnostic tests were found more sensitive as compare to QBC with good negative predictive value (90%). However, in 3 cases the rapid diagnostic test result was false negative, two of these shows grade 1 parasitemia. This may be due to insufficient enzyme production which occurs during early malarial infection or the patient blood samples contained parasites at concentration below the RDT's detection level [11]. Occasional false negative results may be caused by deletion or mutation of the HRP-2 gene [12]. It has been suggested that anti-HRP-2 antibodies in humans may explain why some tests were negative despite significant parasitemia [13]. Presence of an inhibitor in the patient’s blood preventing development of the control line is also noted [14].

False positive RDT results occur in a 12 cases. In 9 of these, *P. falciparum* seen and only in 3, non-*falciparum* detected. This may be explained by the fact that *P. falciparum* can sometimes sequester and may not be present in circulating blood [15]. Cross-reactivity with rheumatoid factor in blood generates a false positive test line, but replacement of IgG with IgM in recent products reduces this problem [16-18]. Cross-reactivity with heterophile antibodies may also occur [19].

In our study, the sensitivity of RDT for *P. falciparum* and *P. vivax* was 96.97% and 87.50% respectively. Palmer et al in 1998 had found a sensitivity of 88% for *P. falciparum* and 94% for *P. vivax* [15].

RDT is a valuable complement to microscopy because it helps expand the coverage of parasite-based diagnosis to the periphery and minimize exclusively clinical diagnosis. RDTs offer a more promising strategy to deal with increasing costs of therapy driven by drug resistance. Today’s multi-million dollar investment in anti-malarial drug development should be accompanied by a parallel commitment to improve diagnostic tools and their availability to those living in malarious areas. RDTs are useful and easy tools for field surveys because they are easily read by the field workers without supervision and require no training or instruments. In situations where adequate laboratory back up is not available, antigen detection test can be employed. However, RDTs may not be able to replace the peripheral smear examination as the most comprehensive and cost-effective test for malaria.
In developing countries, RDTs make obsolete the sole dependence on clinical diagnosis for malaria in remote areas, where good microscopy has failed or never reached. RDTs are also recommended in situations exceeding microscopy capability, such as in an outbreak or in occupationally exposed groups\textsuperscript{20}.

Prompt and accurate diagnosis will not only improve malaria treatment, but possibly reduce morbidity due to other febrile illnesses. Therefore RDTs should be considered as tools for the composite management of febrile illnesses.

5. Conclusions

We concluded that rapid diagnostic test is a simple, sensitive and effective diagnostic test for \textit{P. falciparum} and \textit{P. vivax} in countries like India. The good negative predictive value permits this test to be included as part of a comprehensive diagnostic strategy. However, occasional false-negative results mean that there is a risk of misdiagnosing malaria caused by \textit{P. falciparum}. Although no single test can replace the conventional method of peripheral blood smear examination, RDTs can be useful in areas where specialized laboratories or even microscopy are unavailable and when urgent malaria diagnosis is needed by a practitioner without the delay associated with the laboratory.

6. Reference

2. WHO, Fact sheet no. 94, Recent advances in research and development of malaria, January 2013