Comparison of Different Methods for Diagnosis of Malarial Parasites

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ABSTRACT

The study aims to evaluate the sensitivity and specificity of different methods for diagnosis of malarial infection. Total 200 blood samples were collected in ethylenediaminetetraacetic acid (EDTA) Vacutainer tube from clinically suspected malaria patients. Each sample was processed as thick and thin smear stained with Leishman's stain for light microscopic examination, quantitative buffy coat test and rapid malarial antigen (HRP II and pLDH) test. The detection rate of malarial parasites by microscopic examination was 13.5%, quantitative buffy coat test was 18% and rapid malarial antigen (HRP II and pLDH) test was 20%. Thus, findings of microscopic examination must be compared with other more sensitive methods for confirmation of malaria. This will help in early detection, proper diagnosis and treatment of malaria.

Keywords: Diagnosis, Malarial infection, Quantitative buffy coat, Rapid malarial antigen test.

INTRODUCTION

Malaria is one of the highest killer diseases affecting most tropical countries. It affects over 500 million people worldwide and over one million children die annually from malaria.1,2 According to the United Nations International Children’s Emergency Fund (UNICEF), in every minute, malaria kills a child in the world.3 Of all the human malaria parasites, Plasmodium falciparum (P. falciparum) is most pathogenic and frequently fatal if not treated in time.4 In India, according to Nandwani et al4 a total of 1.82 million cases of malaria and 0.89 million cases of P. falciparum cases were reported in the year 2002. According to National Vector Borne Disease Control Program5 there were 10,66,981 malaria positive cases and 5,33,535 P. falciparum in the year 2012.

The increasing incidence of falciparum malaria, the need to identify and treat the additional infective carriers (reservoirs) and to reduce the chances of transmission has given an impetus for development of simple and rapid methods for the diagnosis of falciparum malaria. Conventional Leishman’s, Giemsa or Romanowsky’s stained peripheral blood examination by light microscopy is the standard method for malaria diagnosis in malaria endemic countries. Conventional light microscopy has the advantages that it is relatively inexpensive, provides permanent record and can be shared with other disease control programs. However, it suffers from disadvantages, such as it is labor intensive and time consuming.6

The purpose of this study was to compare three methods of malaria diagnosis, i.e. microscopic examination of thick and thin blood smear stained with Leishman’s stain, quantitative buffy coat (QBC) test and malascan rapid test for malaria (Pf/Pan Devices) to evaluate the sensitivity and specificity of these tests.

MATERIALS AND METHODS

This prospective study was carried out at Department of Microbiology, Mahatma Gandhi Mission Medical College and Hospital, Kamothe, Navi Mumbai, Maharashtra, India, over a period of 1 year from January 2013 to December 2013.

Study Design

It was a cross-sectional study.

Study Type

It was an prospective and analytical type of study.

Statistical Test

Chi-square test, Z-tests and statistical package for the social sciences (SPSS) (version 17) software was used for statistical analysis.

Inclusion Criteria

A total of 200 samples were collected from clinically suspected cases of malaria of all the age groups in both the sexes attending tertiary care hospital.

Exclusion Criteria

b. In case of malarial parasite smear negative—patients with other positive lab test results—for typhoid fever and dengue fever.

**Ethical Clearance**

Ethical clearance was obtained from the Institutional Ethical Committee of Mahatma Gandhi Mission Institute of Health Sciences (Deemed University), Navi Mumbai, before starting the project.

The detailed history, clinical signs and symptoms were recorded in the proforma. A total of 3 ml venous blood was collected into ethylenediaminetetraacetic acid (EDTA) tube (Becton Dickinson) under sterile precautions. Standard thick and thin smears were prepared. The smears were stained with Leishman’s stain (Lot No. 0000168288-HiMedia Laboratories Pvt Ltd, India) and observed under 100x oil immersion objective lens (Figs 1A to F). The blood collected in EDTA was subjected to QBC test and rapid malarial antigen (RMA) test.

Quantitative buffy coat test was done using QBC kit (Diagnour RFCL Ltd, India). The QBC malaria tube was filled with venous blood up to black marking keeping tube nearly horizontal, rolled tube between fingers several times to mix blood and anticoagulants. Tube was rolled between fingers at least 10 times or for at least 5 seconds to mix blood with coating of acridine orange. Tube was sealed using tube plug and then tube suspender inserted into open end of QBC tube using clean forceps, provided with the kit. Quantitative buffy coat tube was centrifuged immediately. Quantitative buffy coat tube placed on rotor of microcentrifuge and centrifuged at 12000 revolutions per minute (rpm) for 5 minutes (Figs 2A to D).

Malaria plasmodium lactate dehydrogenase (pLDH)/histidine rich protein II (HRPII) was detected according to manufacturer’s instruction using malascan rapid test for malaria (Pf/Pan Devices) (Lot No. V71103Z-Zephyr Biomedicals, India) (Fig. 3).

**RESULTS**

This prospective and analytical study was carried out at Department of Microbiology, Mahatma Gandhi Mission Medical College and Hospital, over a period of 1 year from January 2013 to December 2013. Total 200 blood samples were taken from malaria suspected patients after obtaining informed consent. All samples were tested by three different methods: (a) thick and thin smear made
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From all blood samples and stained with Leishman's stain and microscopic examination was done, 27 samples out of 200, were positive for malarial parasites i.e. 13.5%.

(b) quantitative buffy coat test showed that 36 samples out of 200 were positive for malarial parasites, i.e. 18% and (c) rapid malarial antigen test showed positive result for 40 samples out of 200, i.e. 20% (Table 1 and Graph 1). Actual number of species detected by three methods is shown in Graph 2 and Table 2.

In our study, microscopic findings showed P. vivax 55.56%, P. falciparum 18.52% and mixed species 25.92% (Graph 3). The QBC test showed P. vivax 61.11%, P. falciparum 22.22% and mixed species 16.67% (Graph 4).

Rapid malarial antigen (HRP II and pLDH) test showed P. vivax 62.50%, P. falciparum 25% and mixed species 12.50% (Graph 5).

Statistical analysis of microscopy is Chi-square = 2.12, df=1, p-value = 0.141 and of QBC test Chi-square = 0.177, df=1, p-value = 0.674; p-value of microscopy and QBC test as compared to RMA test are 0.141 and 0.674 respectively nearer to significant p-value of 0.05.

In our study, the sensitivity and specificity of different methods was as follows:

1. Microscopy 67.50% (95% CI: 61.71 to 86.23%) and 100% (95% CI: 97.70 to 100%)

Fig. 2A to D: Detection of malarial parasites by QBC test: (A) Microcentrifuge machine for QBC test, (B) processed QBC tube, (C) para lens advanced w/60x objective attached microscope with para viewer and (D) trophozoite and schizonts of plasmodium vivax along with malarial pigment

Fig. 3: Detection of malarial parasites by RMA test
Table 1: Comparison of different methods for diagnosis of malarial parasite

<table>
<thead>
<tr>
<th>Methods</th>
<th>Malaria positive</th>
<th>Percentages</th>
</tr>
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<tbody>
<tr>
<td>Microscopy</td>
<td>27/200</td>
<td>13.5</td>
</tr>
<tr>
<td>QBC test</td>
<td>36/200</td>
<td>18</td>
</tr>
<tr>
<td>RMA test</td>
<td>40/200</td>
<td>20</td>
</tr>
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</table>

Table 2: Comparison of diagnostic methods for detection of malarial parasites

<table>
<thead>
<tr>
<th>Methods</th>
<th>P. vivax (55.56%)</th>
<th>P. falciparum (18.52%)</th>
<th>Mixed species (25.92%)</th>
<th>Total samples (100%)</th>
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</thead>
<tbody>
<tr>
<td>Microscopy</td>
<td>15</td>
<td>5</td>
<td>7</td>
<td>27</td>
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<tr>
<td>QBC test</td>
<td>22</td>
<td>8</td>
<td>6</td>
<td>36</td>
</tr>
<tr>
<td>RMA test</td>
<td>25</td>
<td>10</td>
<td>5</td>
<td>40</td>
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DISCUSSION

There are reports or studies on diagnosis of malaria by different methods from India and other countries. There are some differences depending on geographic location, disease burden, endemicity and methods followed.

Total 200 blood samples of suspected malaria cases were studied by different methods. Microscopy, QBC test and RMA test showed positivity of malarial parasites as 13.5, 18 and 20% respectively in our study (Table 1).
Our results of microscopy compared well with Mendiratta DK et al 6 (18.28%) and Aparna Y et al 8 (13.87%). Azikiwe CCA et al 7 from Nigeria reported microscopic finding of malaria as 59%, Ali-Akbar et al 9 from Kahnuj, Iran found sensitivity of 77% by microscopy sensitivity of 77% by microscopy (Table 3).

The reasons for low sensitivity of microscopy are:
1. It is affected by time of blood collection. Malarial parasites will be seen only when blood sample is collected at the time of febrile episode.
2. Lot of subjective variation. Many false positives and false negatives.
3. Prior treatment with antimalarial can lead to negative results in microscopy.

Reporting of malarial parasites by microscopy is subjective and many variations can occur in results from person to person. Some over or under reporting can occur. Similarly, the higher values are reported from highly endemic areas like Africa.

Our results of QBC test (18%) correlate well with Aparna Y et al 8 (20.44%). Other workers do not have data for QBC test. Quantitative buffy coat test is more sensitive than light microscopy as the blood sample is centrifuged at 12,000 rpm and RBCs are concentrated. Positivity of RMA test in our study (20%) is close to Mendiratta DK et al 6 (18.1%), Aparna Y et al 8 15.33%. Azikiwe CCA et al 7 reported higher positivity of RMA test (64%) as compared to microscopy, which is similar to our findings. Sensitivity of RMA test was higher than light microscopy and QBC test. As mentioned in review, light microscopy and QBC test detect the parasites in red blood cells where as RMA test can detect the malarial antigen in plasma which is released by analysis of RBCs in blood, in patient (in vivo) and then in the test procedure (in vitro).

As regards, species wise detection of malarial parasites by different methods, in our study, microscopic findings were P. vivax 55.56%, P. falciparum 18.52% and mixed species 25.92% (Graph 3). In QBC test findings, positive results were P. vivax 61.11%, P. falciparum 22.22% and mixed species 16.67% (Graph 4). In RMA (HRP II and pLDH) test, findings out of 40 were P. vivax 25% and mixed species 12.50% (Graph 5).

The results show that with RMA test which is based on detection of malarial antigens (HRP II and pLDH), the results of mixed species by microscopy decreased from 7/27 (25.92) to 5/40 (12.5%). Microscopy is subjective and there is possibility of over reporting of mixed malarial parasitic infection.

Statistical analysis of microscopy is Chi-square = 2.12, df = 1, p-value = 0.141n and of QBC test Chi-square = 0.177, df = 1, p-value = 0.674.

p-value of microscopy and QBC test as compared to RMA test are 0.141 and 0.674 respectively nearer to significant p-value of 0.05.

CONCLUSION

Quantitative buffy coat test and RMA test are more sensitive than microscopy. Rapid malarial antigen being immunological method (detecting HRP II antigen and pLDH enzyme) has high sensitivity and specificity, easy to perform. Quantitative buffy coat test is less sensitive than RMA test requires specialized equipment and training. Light microscopy is least sensitive and subjective. Hence, when microscopic examination is negative, it is necessary to perform RMA test, which has high sensitivity and specificity. This will help early detection of malaria, proper diagnosis and treatment, reducing morbidity and mortality.

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REFERENCES


