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Original article:

Study of accuracy of detection of malaria parasite in clinically suspected cases by PBS, MCBS and antigen detection methods

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Abstract:

Introduction: The earliest symptoms of malaria are very nonspecific and variable such as fever, headache, body ache, malaise, fatigue and abdominal discomfort. The laboratory diagnosis of malaria is done by different techniques such as the conventional thin and thick peripheral blood smears (PBS), concentration techniques such as centrifuged buffy coat smears (CBCS) and fluorescent (QBC ®) technique, Serologic tests such as the detection of parasite-specific proteins' Rapid Diagnostic Tests (RDT) and Polymerase Chain Reaction (PCR).

Material and methods: The present study was done in a tertiary care hospital for two years duration. 328 patients with symptoms suspicious of malaria (fever with chills, headache and nausea and vomiting) were enrolled in the study. Blood from 200 other patients, who had no clinical suspicion of malaria, was also taken and they acted as controls. MCBS test was done alongwith other tests to assess its comparative advantage over other methods.

Results: In the present study out of 146 cases, 95 (65.06%) cases were diagnosed on smear out of which 76 (68.85%) were P. vivax and 17(54.54%) were P.falciparum. 1 case was diagnosed as having mixed infections.

Conclusion: The Peripheral Blood Smear method (PBS) is a well established gold standard in malaria diagnosis since many years. It is very specific and useful in differentiating species and quantification of parasitemia. However low sensitivity in comparison with other methods limits its usefulness and there is a large percentage of smear negative patients. Also it is cumbersome to use and needs specially trained personnel to interpret the smears.

Introduction:

Malaria caused 67321 cases and 164 deaths in Maharashtra in year 2008[1]. The earliest symptoms of malaria are very nonspecific and variable such as fever, headache, body ache, malaise, fatigue and abdominal discomfort. Hence, there is difficulty in clinically diagnosing malaria but the treatment has to be started immediately in order to avoid complications. [2]The nonspecific nature of the clinical presentation of malaria may lead to over-treatment of malaria in malaria endemic areas and missing the diagnosis of malaria in low-transmission areas. Therefore precise laboratory diagnosis and species identification is very essential.[3]

The laboratory diagnosis of malaria is done by different techniques such as the conventional thin and thick peripheral blood smears (PBS), concentration techniques such as centrifuged buffy coat smears (CBCS) and

fluorescent (QBC ®) technique, Serologic tests such as the detection of parasite-specific proteins' Rapid Diagnostic Tests (RDT) and Polymerase Chain Reaction (PCR).

Material and methods:

The present study was done in a tertiary care hospital for a period of one year. 328 patients with symptoms suspicious of malaria (fever with chills, headache and nausea and vomiting) were enrolled in the study. Blood from 200 other patients, who had no clinical suspicion of malaria, was also taken and they acted as controls.From each of the 328 patients, detailed clinical history including age, sex, presenting complaints was taken. 2 ml of venous blood was collected in an EDTA bulb, subjected to three techniques for diagnosis of malaria; peripheral smear examination (PBS), Modified Centrifuged Blood Smear (MCBS) and Antigen detection (dipstick method). The patient was declared to be having malaria if he was positive by any one of the methods employed and was called a CASE. The blood was also subjected to analysis by a three part hematology analyzer (ERMA PCE 210) and Hb value, total WBC count, platelet count and RDW were noted. Differential count was done by peripheral smear examination as our three part counter could not differentiate between eosinophils, monocytes and basophils. Similarly blood from 200 patients negative for malarial symptoms was subjected to assessment of the same hematological parameters. They were tested for malaria by all the three methods as well.

Results:

Table 1 Analysis of methods used for detection of malaria

Method	Sensitivity	Specificity	PPV	NPV	kappa coefficient of agreement
PBS	63.33	99.45	98.95	78.44	0.67
MCBS	77.93	99.45	99.12	85.04	0.77

Table 7

Anemia distribution

(Normal range[126] – males 13-17gm%, females – 12-15 gm %)

<table>
<tr><th></th><th>MALES Hb < 13 gm%</th><th>MALES with normal Hb (>13 gm%)</th><th>FEMALES Hb <12 gm%</th><th>Females with normal Hb (12 gm%)</th><th>Total</th></tr>
<tr><td>PV</td><td>35</td><td>54</td><td>17</td><td>6</td><td rowspan="3">146</td></tr>
<tr><td>PF</td><td>19</td><td>8</td><td>6</td><td>0</td></tr>
<tr><td>MIXED</td><td>1</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>CONTROLS</td><td>36</td><td>95</td><td>27</td><td>42</td><td>200</td></tr>
</table>

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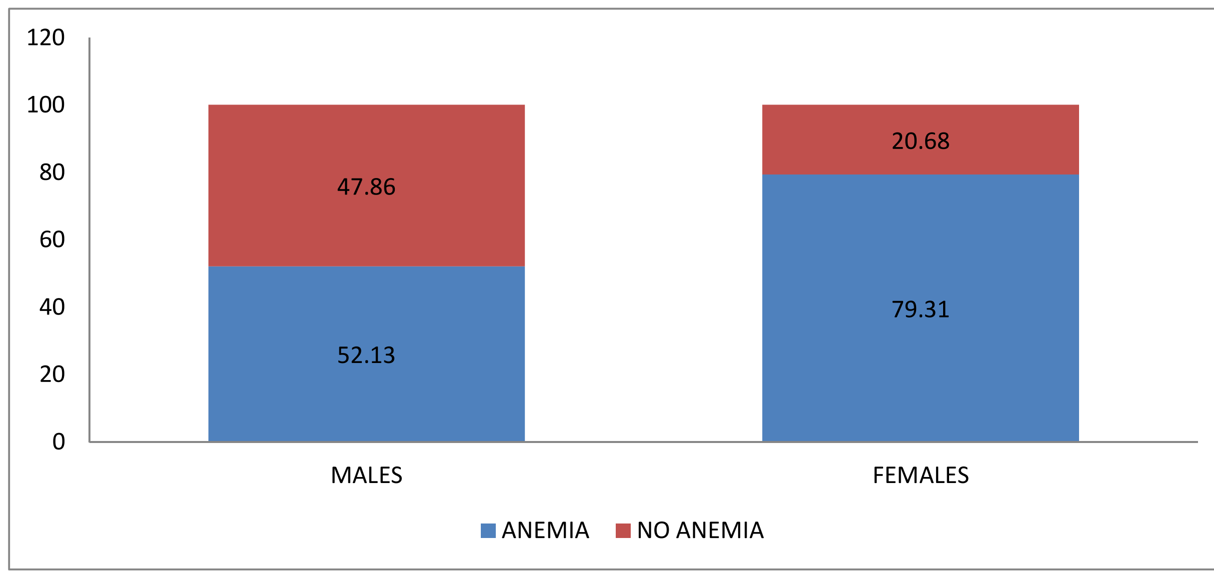
Graph 1 **Percentage of patients showing anemia**



We saw that prevalence of anemia was higher in females than males by a ratio of 1.52:1 and higher in P. falciparum than P. vivax malaria by a ratio of 2.02:1. The association of anemia with malaria was highly significant ($p<0.05$).

Discussion:

Microscopic examination remains the "gold standard" for laboratory confirmation of malaria[4]. It is an established, relatively simple technique that is familiar to most laboratorians. Any laboratory that can perform routine hematology tests is equipped to perform a thin and thick malaria smear. Within a few hours of collecting the blood, the microscopy test can provide valuable information. First and foremost it can determine that malaria parasites are present in the patient's blood. Once the diagnosis is established the laboratorian can examine the thin smear to determine the malaria species and the parasitemia, or the percentage of the patient's red blood cells that are infected with malaria parasites. The smears are able to provide all 3 of these vital pieces of information to the doctor to guide the initial treatment decisions that need to be made acutely[5]. However, microscopic parasitological diagnosis requires continued personnel training and supervision in addition to a minimum laboratory structure. Additionally, such a test is prone to large observer-related variation and lacks sensitivity when performed by non-expert laboratory microscopists[5].

In the present study out of 146 cases, 95 (65.06%) cases were diagnosed on smear out of which 76 (68.85%) were P. vivax and 17(54.54%) were P.falciparum. 1 case was diagnosed as having mixed infections. The percentage of positivity by peripheral smear in our study was concordant with other studies as shown in the following table. The antigen detection kit available at our institute was provided by Aspen Laboratories (Malarigen) and comprised of Pan specific Aldolase (P. vivax and P. falciparum) and P. falciparum specific pLDH. Our

laboratory data indicated that of P. vivax and P. falciparum were the only two prevalent species and predominant species was of P. vivax, hence the kit was accordingly suitable to be used.

The most studied malaria Rapid diagnostic tests (RDTs) offer simple identification of two parasite antigens: histidine-rich protein 2 (HRP2) and plasmodium lactate dehydrogenase (pLDH). HRP2 was the first antigen targeted by an RDT[6], has been available in various commercial formats for several years, has shown good sensitivity in a variety of field settings, and is increasingly advocated as a diagnostic test where reliable microscopy is not available. A potential problem for HRP2-based assays is persistence of detectable circulating antigen for up to several weeks after parasites have been eradicated. Persistent HRP2 antigenemia has not correlated with treatment failure, suggesting that this finding is not representative of persistent infection. Persistent antigenemia thus may limit the usefulness of HRP2-based assays in areas of intense malaria transmission, where positive tests may commonly be due to prior infections that are no longer clinically relevant.[7]

Conclusion:

The Peripheral Blood Smear method (PBS) is a well established gold standard in malaria diagnosis since many years. It is very specific and useful in differentiating species and quantification of parasitemia. However low sensitivity in comparison with other methods limits its usefulness and there is a large percentage of smear negative patients. Also it is cumbersome to use and needs specially trained personnel to interpret the smears.

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