

A study to correlate smear microscopy and rapid antigen detection techniques in the diagnosis of malaria

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Introduction: Malaria is an endemic, tropical protozoan infection caused different Plasmodium species. **Materials and Methods:** Study was conducted in the Department of Microbiology, GSL Medical College for 6 months. Peripheral blood smear stained by Giemsa technique and commercially available rapid antigen detection kits were used for the diagnosis of malaria. **Results:** Among 98 the clinically diagnosed malaria cases in this study, 69 (70.4%) cases were identified as malaria positive. Out of these, 68% (47) were positive by blood smear examination and 87% (60) were positive by kit method and in the positive cases 35 were male and 34 were female participants. **Conclusion:** Rapid diagnostic tests were more sensitive in the diagnosis of malaria. However, other confirmatory tests such as PCR should be considered because some clinically proved cases were missed.

Keywords: Among, Blood smear, Malaria, Plasmodium

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Introduction

Malaria is an endemic, tropical protozoan infection caused different Plasmodium species. *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium ovale* and *Plasmodium knowlesi* are the members of plasmodium that cause malaria [1, 2]. Malaria is a serious infection, leads to death if not treated properly. As per the available reports, worldwide, the prevalence of malaria was reported as 300 million per annum [3, 4]. In malaria, symptoms such as fever, rigors, anemia, enlargement of spleen, liver and

So on were reported. Among the members, of plasmodium members, malaria caused by *P. falciparum* is severe, leads to death especially in children due to involvement of brain, cerebral malaria. But malaria caused by *P. vivax* was considered to be mild but leads to death. As per the recent studies, the global death rate due to vivax malaria was reported to be 0.1- 1.6% [5]. Due to large number of cases and deaths, government of India inaugurated anti malaria programme in 1953, Different diagnostic methods such as peripheral blood smear, serodiagnostics, antigen demonstration, molecular methods being practiced for the diagnosis of this killer

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Parasitic infection. Several advantages and disadvantages were reported for these techniques. With these, a study was conducted to find the correlation between the peripheral blood smear and antigen demonstration for the detection of malaria.

Materials and Methods

Settings: Study was conducted in the department of Microbiology, GSL Medical College.

Duration of study: Study was conducted for 6 months, September 2018 to February 2019.

Sampling method: Random sampling was considered in this study.

Inclusion criteria: Individuals clinically diagnosed as malaria, aged >18 years, who submitted informed consent, were included in the study.

Exclusion criteria: Individuals aged <18 years, who didn't submitted informed consent, were excluded in the study.

Sample size: All the individuals who satisfy the inclusion criteria during the study period were included in the study.

Finger prick technique was used to collect blood for peripheral smear. Two new, grease free glass slides were used for thick and thin smear preparation, Giemsa stain was used to the smears as per the WHO guidelines [7, 8]. The stained smears were examined under 100X objectives using light microscope. A minimum of 100 to 200 fields were screened for the diagnosis of malaria. Smears were screened for the detection of various morphological stages inside the RBC such as ring forms, gametocytes.

Commercially available, rapid malaria Pan+Pf cards were used for antigen. First, the test kits were brought to the room temperature before the procedure and. As per the manufacture guidelines, by using sample dropper provided with the test kit, whole blood was pipetted up to the mark and dispensed on the sample well S. this step was followed by adding three drops of buffer to the well B. Then the results were read after 20 minutes time. Presence of three purple to pink lines in the regions of F, P and C indicated that the sample was reactive for *P. falciparum*, *P. vivax* and other species like *P. malariae* and *P. ovale*. Whereas, band at C and F region indicated that the sample was positive only for *P. falciparum*. If the bands were seen on C and P, the sample was considered positive to all other Plasmodium species. Presence of one band on C region even after 20 minutes indicates the test is negative.

Results

Among 98 the clinically diagnosed malaria cases in this study, 69 (70.4%) cases were identified as malaria positive. Out of these, 68% (47) were positive by blood smear examination and 87% (60) were positive by kit method (Table 1).

Table-1: Comparison of the techniques.

Technique	Positive	%	Cost per test in rupees
Blood smear	47	68	10-15
Kit method	60	87	100

In this study total 69 samples were diagnosed to be malaria. In this 35 were male and 34 were female participants, with male female ratio 35:34. When ages of the participants was considered, 10 were positive in 18-28 age group, 16 were positive in 29-39 years age group, 11 were positive in 40 – 50 years age group, 15 were positive in 51-60 age group and 17 were positive in >61 years age group. Whereas when gender was considered, among male, 7 were positive in 18-28 age group, 9 were positive in 29-39 years age group, 5 were positive in 40-50 years age group, 8 were positive in 51-60 age group and 6 were positive in >61 years age group. Whereas among female, 3 were positive in 18-28 age group, 7 were positive in 29-39 years age group, 6 were positive in 40-50 years age group, 7 were positive in 51-60 age group and 11 were positive in >61 years age group (Table 2).

Table-2: Age wise test results culture result among nondiabetic individuals; n (%).

Age	Male	Female	Total
18-28	7	3	10
29-39	9	7	16
40-50	5	6	11
51-60	8	7	15
> 61	6	11	17
Total	35 (51)	34 (49)	69 (100)

Discussion

Malaria diagnosis has to be considered to be health emergency and there should not be any delay in the diagnosis. But the malaria diagnosis is a significant challenge in the medical field [2, 9]. Smear microscopy is commonly practiced technique not only for the diagnosis but also treatment monitoring, to note the drug resistance of malaria. In most of the settings, perheral blood smear reared from fingertip blood is being used for the diagnosis of malaria. But requirement of prolonged time

Period, technical expertise are the major draw backs. In spite of these draw backs, low cost is the major factor for considering smear microscopy for the diagnosis of malaria in the study. Several reports mentioned that requirement of trained microscopist and prolonged time period are the major draw backs of this technique. But in tertiary health care setup, manpower is not an issue because every institution has to recruit the manpower as per the norms. Moreover, various paramedical as well as nursing students are the additional asset where the majority work is being carried by them as a part of their training. Hence manpower is not an issue in tertiary health care units especially in India. In this study, 68% (47) cases were diagnosed as malaria by using peripheral blood smear (Table 1). Whereas it was reported to be 75% and 62% [11,12]. Coleman RE et al., quoted that microscopy is the gold standard technique for the diagnosis of malaria [13]; In spite of this the authors reported 10% false positive results [14, 15, 16]. In another report by Abisha Jaya Singh et al., the sensitivity was reported to be 75%; but the specificity as well as positive predictive values were reported to be 100% for microscopy [17]. Whereas Gulrez et al., [11] reported that the peripheral blood smear positivity was 61%. The hope behind the development of rapid malaria diagnostic technique was rapidity, low price and accuracy [18]. The rapid diagnostics were also proved to be the backbone for the diagnosis of malaria especially in the areas where the microscopic facilities are not available [8,19]. In this report, the antigen detection by kit methods detected 87% (60) cases. Santhy et al., reported that the malaria positivity with the kit methods was 52.4%; but the specificity was reported to be 85% and PPV was 62.5% [10]. The investigators reported that smear and antigen detection have similar specificity and PPV [17]. The range of sensitivity with kit method was ranged 76 – 98% [20]. The findings of the present study were similar to the available reports [21]. There were 19 cases missed by the peripheral smear microscopy. Among the missed cases, the cause was identified to be *P. falciparum* by the rapid diagnostic tests. As per the reports, this could be due to sequestration which is the important virulence in case of *P. falciparum* [22]; this is nothing but holding back of the parasite in the capillaries of the internal organs. When gender was considered, 51% (35) and 49% (34) cases were diagnosed as malaria positive respectively for male and female category. Abisha

Jaya Singh et al., reported that the 80% and 20% cases were diagnosed to be malaria respectively in the gender [17]. It was reported in the literature that more outdoor activity is the major cause for more positive results in the male group. But all the present study participants had outdoor activity because most (85%) of these were daily labor and the remaining were employees. So surely the exposed to the bite of anopheles mosquito. Due to this outdoor activity, the malaria positivity was similar in the gender. Age wise, malaria positivity was increased with the age in this study; this is also due to more outdoor activity.

Limitations of the study: Small sample size, short duration of the study are the limitations of the study.

Conclusion

Rapid diagnostic tests were more sensitive in the diagnosis of malaria. However, other confirmatory tests such as PCR should be considered because some clinically proved cases were missed.

What the study adds to the existing knowledge?

The present study aimed to establish the sensitivity in the diagnosis of malaria in GSL Medical College for 6 months, however the study also supports the fact that far more inclusive test are required such as PCR to determine the diagnosis in a broader manner.

Authors contributions

Dr. Khatija Shameem: Literature survey, data analysis, paper writing, **Dr. T Jaya Chandra:** Sample collection, Bench work, statistical analysis, paper writing

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