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Original Research Article

Study of malaria in febrile patients attending tertiary health care center and evaluation of peripheral smear examination, quantitative buffy coat and rapid diagnostic test in the diagnosis of malaria

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ABSTRACT

Background: The objective was to study the prevalence of malaria in febrile patients attending the hospital and to evaluate peripheral smear examination, quantitative buffy coat and rapid diagnostic test in the diagnosis of malaria.
Methods: The study group includes 208 patients presenting with fever with chills and rigor and other suggestive symptoms of malaria attending the tertiary care center. 5 ml of venous blood was collected in ethylene diamine tetra acetate (EDTA) tube from each patient and the samples were processed for peripheral blood smear examination, quantitative buffy coat (QBC) and antigen detection by rapid diagnostic test (RDT).
Results: Out of 208 suspected cases of malaria, 3 (1.44%) were positive for malaria and 205 (98.55%) were negative, the prevalence of malaria was found to be 1.44%. Of total 208 cases tested, 3 cases (1.44%) were positive for malaria by peripheral blood smear examination and by QBC and with rapid diagnostic test only 2 cases (0.96%) were positive. *P. vivax* was detected in all 3 positive cases.
Conclusions: Peripheral smear examination is considered as gold standard method for diagnosis of malaria. QBC can be helpful when an experienced microscopist is not available. Rapid diagnostic tests are simple, rapid, do not need expertise, interpretation of results is easy and objective and useful in routine diagnosis.

Keywords: Malaria, Peripheral blood smear examination, Quantitative buffy coat, Rapid diagnostic test

INTRODUCTION

Malaria is one of the important endemic disease of tropics and subtropics and is a major public health problem worldwide. It can cause death if not diagnosed and treated early. Even with much advances in the treatment and prevention of malaria, malaria is still a threat to lives of millions of people.[1] According to the latest world malaria report, there were 241 million cases and 627000 deaths globally in 2020. African region of the World Health Organization is account for over 95 percent of the burden, followed by 2 percent each in the South East Asian and Eastern Mediterranean regions, with the American and Western Pacific regions contributing the remainder.[2] In the South East Asian region, India contributes to 83% of the cases.[2]

In India, malaria cases have constantly declined from 2.09 million (20.9 lakhs) to 0.19 million (1.9 lakhs) during 2001 to 2020 and during the same period *Plasmodium falciparum* cases have declined from 1 million (10 lakhs) to 0.12 million (1.2 lakhs) cases.[1] National Vector Borne Disease Control Programme (NVBDCP) has currently reported in the year 2021 that in India there are 1.58 lakhs cases of malaria and 99,239 cases of *Plasmodium falciparum*.[3]

Malaria is a fatal disease caused by the infection of red blood cells with protozoan parasites of the genus

Plasmodium and is transmitted to people by the bite of infected female Anopheles mosquitoes.[1] There are four species of Plasmodium which are most commonly infect humans namely *P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale*. *P. vivax* and *P. falciparum* are the most common species and *P. falciparum* is the most threatening.[1] One of the reasons for occurring of 95% of the world's malaria cases in Africa could be due to the strong human-biting habit of the African vector species.[1]

There is difficulty in diagnosing malaria clinically but the treatment has to be started immediately in order to avoid complications. Hence, for effective management of malaria prompt and accurate diagnosis of the condition is essential.

The commonly available diagnostic tests for malaria are peripheral blood smears examination, quantitative buffy coat (QBC) and rapid diagnostic tests (RDT).[4] Peripheral blood smears examination is the most commonly used method for detection of malaria parasites and it is the gold standard for diagnosis of malaria and is easily available and has low cost.[4] Quantitative buffy coat (QBC) in which malaria parasite is detected using the fluorescent dyes which stains the nucleic acid of the malarial parasite. Rapid diagnostic test (RDT) detects antigens of malaria parasite such as plasmodium lactate dehydrogenase (pLDH), histidine rich protein-2 (HRP 2), and pan-specific aldolase.[4] Rapid card test are commonly used and more sensitive in detecting malaria parasite. All these malarial diagnostic tests techniques differ in their sensitivity, specificity, positive and negative predictive values.[4]

Hence, the present study was undertaken to study the prevalence of malaria in febrile patients attending the hospital and to evaluate peripheral smear examination, quantitative buffy coat and rapid diagnostic test in the diagnosis of malaria.

METHODS

This prospective study was conducted in the department of microbiology, Adichunchanagiri Institute of Medical Sciences, BG Nagara, Karnataka, India from December 2021 to July 2022.

Inclusion criteria

Patients of all ages presenting with fever with chills and rigor and other suggestive symptoms of malaria were included in the study.

Exclusion criteria

Patients having fever with chills with obvious focus of infection like abscess, urinary tract infection (which also causes fever with chills) and patients who had received treatment for malaria within past 4 weeks were excluded from the study.

208 patients satisfying the inclusion criteria were selected and samples were collected. The study was approved by the Institutional Ethical committee. Consent was obtained from all patients.

Sample collection

Informed consent has been taken from the patient prior to the sample collection. 5 ml of venous blood was collected in ethylene diamine tetra acetate (EDTA) tube from each patient and sample transported and processed immediately in the laboratory. The samples were processed for peripheral blood smear examination, quantitative buffy coat **(**QBC) and antigen detection by rapid diagnostic test (RDT).

Thick and thin blood smears

Thick and thin blood smears were prepared as per the standard method. The smears were stained with Leishman's stain. A total of 200 to 300 oil immersion fields were examined before the smear was declared negative.[5]

Quantitative buffy coat (QBC) technique

The principal involves staining of nucleic acid of malarial parasites with acridine orange in centrifuged and compressed red cell layer and its examination under fluorescent microscope. In the QBC technique, 65 µl of blood was taken in a specialized capillary tube coated internally with acridine orange and anticoagulant. Tube was closed with closure, float was inserted inside the tube and then centrifuged in the QBC microhematocrit centrifuge at 12,000 rpm for 5 minutes. Centrifugation concentrates parasitised red cells around float and buffy coat in centrifuged capillary tube was examined under fluorescent microscope. Malaria parasites which were stained by acridine orange appeared brilliant green.[6]

Antigen detection by RDT

Malaria antigen was detected by immunochromatographic (ICT) method with malaria P.f/Pan Ag malaria antigen test kit (SD Biosensor). Test was performed as per the instructions given in the kit insert. Malaria P.f/Pan Ag qualitatively detects *Plasmodium falciparum* and Plasmodium species antigens in whole blood using immunochromatography. The target antigen is Histidine-Rich Protein II (HRP-II) of *Plasmodium falciparum* and plasmodium lactate dehydrogenase (pLDH) of Plasmodium species.[7]

RESULTS

In the present study, blood samples were collected from 208 patients having fever with chills and rigor and other suggestive symptoms of malaria.

Table 1: Sex distribution.

Sex	Number of patients	Percentage
Males	127	61
Females	81	39
Total	208	100

Table 2: Age distribution.

Age (years)	Number of patients	Percentage
1-10	3	1.44
11-20	11	5.28
21-30	74	35.57
31-40	49	23.55
41-50	18	8.65
51-60	28	13.46
61-70	25	12
Total	208	100

Of 208 cases, 127 (61%) were males and 81 (39%) were females (Table 1). Age distribution of the patients includes 3 (1.44%) were in the age group of 1-10 years, 11 (5.28%) of 11-20 years, 74 (35.57%) of 21-30 years, 49 (23.55%) of 31-40 years, 18 (8.65%) of 41-50 years, 28 (13.46%) of 51-60 years and 25 (12%) of 61-70 years respectively. Majority of the patients were in the age group of 21-30 years (Table 2).

Table 3: Positivity for malaria.

Results	Number of samples	Percentage
Malaria positive	3	1.44
Malaria negative	205	98.55
Total	208	100

In the present study, out of 208 suspected cases of malaria, 3 (1.44%) were positive for malaria and 205 (98.55%) were negative, the prevalence of malaria was found to be 1.44% (Table 3).

Table 4: Comparison of peripheral blood smear examination, QBC and rapid diagnostic test.

Results	Peripheral blood smear examination	QBC	Rapid diagnostic test
Positive	3 (1.44%)	3 (1.44%)	2 (0.96%)
Negative	205 (98.55%)	205 (98.55%)	206
Total	208	208	208

Of total 208 cases tested, 3 cases (1.44%) were positive for malaria by peripheral blood smear examination and by QBC and with rapid diagnostic test only 2 cases (0.96%) were positive. *P. vivax* was detected in all 3 positive cases (Table 4)

DISCUSSION

Malaria is a major public health problem worldwide and can cause death if not diagnosed and treated early. In order to avoid complications due to malaria and for the effective management of malaria, prompt and accurate diagnosis is essential.[1] Newer techniques like QBC and antigen detection tests are rapid, simple and easy to interpret.

In the present study, majority of the patients were in the age group of 21-30 years (35.57%) which was similar to other studies conducted elsewhere indicating active working group who are exposed to the mosquito bites especially in the fields and outdoors.[8,9]

In our study, out of 208 suspected cases of malaria, 3 (1.44%) were positive for malaria and 205 (98.55%) were negative and the prevalence of malaria was found to be 1.44% which was lower comparative to other studies and this could be due to improved diagnostic methods and preventive measures.[3]

In this study, out of 208 cases, 3 cases (1.44%) were positive for malaria and 205 cases (98.55%) were negative by peripheral blood smear examination, the blood smear positivity was 1.44%, which was comparable with Ndao et al and Mendiratta et al.[10] In all 3 malaria cases *P. vivax* was detected. In our study, *P. vivax* was the major parasite causing malaria. In India, 70% of the infections are reported to be caused by *P. vivax*.[3] The peripheral blood smear examination is considered to be the gold standard method for diagnosis of malaria but its disadvantages are interpretation of smear is laborious, time consuming and needs an expert microscopist and sensitivity is less in cases of low parasitaemia.[11]

In the present study, of the 208 cases tested by QBC, 3 cases (1.44%) were tested positive for malaria and 205 cases (98.55%) were tested negative, the QBC positivity was 1.44%. The major advantages of QBC method over peripheral blood smear examination are it is simple, highly sensitive, its speed, easy to interpret and to detect gametocytes of *P.vivax and P. falciparum* and also ability to detect low levels of parasitaemia.[11] The limitations of QBC method are expensiveness, false positive results could be due to artifacts such as cell debris and difficult to speciate the ring forms.[11] The sensitivity, specificity, positive predictive value (PPV) and negative predictive values (NPV) of our study by QBC method were 94.6%, 100%, 100% and 96.9% respectively. The sensitivity,

specificity, PPV and NPV values of present study were consistent with other studies.[12]

In our study, of the 208 cases tested for malarial antigen by Malaria P.f/Pan Ag malaria antigen test kit (RDT-SD Biosensor), 2 (0.96%) cases were positive for malaria by antigen detection and 206 (99%) cases were negative, the RDT positivity was 0.96%. One malaria case which was negative by RDT (pLDH) was found to be positive by peripheral blood smear examination and QBC. This could be due to low antigen levels or insufficient enzyme production which occurs in early malarial infection.[12] The major advantages of the rapid diagnostic test are it is user friendly and interpretation of results are easy and is more objective as compared to smear examination and QBC.[13] The sensitivity, specificity, PPV and NPV values of present study are consistent with other studies.[11,13]

CONCLUSION

Malaria is an endemic in certain regions of India, there is a need to use more sensitive tests, which can detect low levels of parasitemia in population. Peripheral smear examination is considered as gold standard method for diagnosis of malaria as it has the advantage of high sensitivity, accurate speciation and quantifiable results. QBC can be helpful when an experienced microscopist is not available. Rapid diagnostic tests are simple, rapid, do not need expertise, interpretation of results is easy and objective and useful in routine diagnosis and in situations where adequate laboratory facilities are not available.

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