ORIGINAL RESEARCH PAPER

Volume-7 | Issue-5 | May-2018 | PRINT ISSN No 2277 - 8179

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

COMPARATIVE STUDY OF PERIPHERAL BLOOD SMEAR WITH QBC AND RAPID DIAGNOSTIC TEST IN SCREENING OF MALARIA

Microbiology

Luxmi Singh M.Sc. Student, Department of Microbiology, Teerthanker Mahaveer Medical College and Research center, Moradabad, Uttar Pradesh, India.

Sudhir Singh* Assistant Professor, Department of Microbiology,Teerthanker Mahaveer Medical College and Research center, Moradabad, Uttar Pradesh, India. *Corresponding Author

Umar Farooq Professor & HOD, Department of Microbiology,Teerthanker Mahaveer Medical College and Research center, Moradabad, Uttar Pradesh, India.

ABSTRACT

Background: Malaria is a life threatning disease caused by plasmodium species of protozoa, it is estimated that 2-3 million deaths occur annually due to malaria, out of which 70-80 thousand death only occur in India.

Material and Method: 200 samples were included in the study, which were further processed for peripheral blood smear (PBS), QBC and Rapid diagnostic test to diagnose malaria.

Result: A total 200 samples were tested by Peripheral blood smear (PBS) in which 65 (32.5%) were positive cases, in QBC (quantitative buffy coat) technique 60 (30%) were positive and Antigen card Test (ACT) 70 (35%) positive cases in malaria. It comparison of PBS with QBC and RDTs, the sensitivity and specificity were 91.66%, 96.42% and 92.30%. 93.10%.

Conclusion: Malaria is a serious health issue which required immediate treatment. We concluded PBS has sensitive & specific compared to QBC and RDTs.

KEYWORDS

Malaria, PBS, QBC, ACT

Introduction:

Malaria is a mosquito-borne protozoal disease caused by infection with parasites of the genus Plasmodium. It is transmitted to man by infected female Anopheles mosquito. People with malaria often experience fever, chills, and flu-like illness. Approximately 156 named species of plasmodium which infect various species of vertebrates but in human there are four known species of *P. falciparum, P. vivax*, *P.malariae* and *P.ovale* are infected [1]. The species *P. knowlesi* rarely causes disease in human. It is found more than 200 million cases and 1.1-2.2 million deaths due to malaria every year. After untreated, they may develop severe complications. In 2016 an approximately 216 million cases of malaria occurred worldwide and 445,000 people died, mostly children in the African Region. About 1,700 cases of malaria were diagnosed in the United States each year[2].

In India malaria is a serious disease posing a major public health. Approximately 0.32 million cases found and about 335 deaths every year in India[3]. Malaria due to *P. falciparum* is fatal due to multi system disease associated with complication. About 1.8 million cases were reported, in which 44.5% cases were cause by *P. falciparum* year 2005 in India. [4] Microscopic examination of peripheral blood smear is the standard method for malaria diagnosis, which is easily available and has low cost. Rapid card test are widely used and are more sensitive in detecting malaria parasite.QBC technique is one of them in which malaria parasite is detected using the fluorescent dyes.

Malaria possesses diagnostic challenge to the medical community worldwide. There is difficulty in diagnosing malaria clinically but the treatment has to be started immediately in order to avoid complications. The nonspecific nature of the clinical demonstration of malaria may lead to over-treatment of malaria in endemic areas and missing the diagnosis of malaria in low-transmission areas. Hence, accurate diagnosis and species identification is very essential. [5]

Material and Method:

A total of 200 samples were collected from patients with sign and symptoms suggestive of malaria attending Teerthanker Mahaveer Hospital and Research Center, Moradabad, during the period 12 month from February 2017 to January 2018. The samples were processed for PBS, QBC and ACT. The study was conducted in parasitology section of microbiology department .

Collection of blood sample

Sample were collected from clinically suspected patient under aseptic condition, from each patient 5 ml of blood sample was collected in Ethylene Diamine Tetra Acetic acid (EDTA) vial.

Peripheral Blood Smear (PBS)

Thick and thin blood film were made on clean glass as per standard procedure were stain with Leishman's stain and microscopically examination for malaria parasite under oil immersion objective. A total of 200to 300 microscopic fields were examined before the film was declared negative.[6].

Quantitative Buffy Coat (QBC) technique

In the QBC technique, approximately 55-65 µl of blood was taken into a capillary tube coated with acridine orange, Potassium oxalate and fitted with a cap. A plastic float was inserted inside the tube and spun in the QBC microhematocrit centrifuge at 12,000 rpm for 5 minutes. The principle of QBC technique is based on the fact that on centrifugation at a high speed, the whole separates into plasma, buffy coat and packed red cell layer. The float gets buoyed by the packed blood cells and is automatically positioned within the buffy coat layer. Blood cells in the buffy coat layer separate according to their densities, forming visibly discrete bands. Due to acridine orange, the malaria parasite stains green (nucleus) and orange (cytoplasm). The tube is examined in the region between the red blood cells and granulocytes and within the granulocytes and mononuclear cell layer, where the parasites are more abundant.[7]

Malaria Rapid Diagnostic Test (RDTs)

RDTs based on immunochromatographic method to detect antigen derived from malaria parasite in lysed blood. Tests are commercially available. Malaria HRP2/ pLDH (Pf/Pv) combo has been tested with clinical sample.[8]

Result

The study was carried in TMMC & RC. hospital, Moradabad UP. Total 200 blood samples were collected from the cases with symptoms of malaria. Out of 200 patients 110 (55%) were male and 90 (45%) were female (Table 1). Maximum number of patient belong to age group 21-30 year followed by 41-50 year ,11-20,51-60 (Table 2). Out of 200 samples 65 were smear positive and 135 were smear negative (Table 3). In smear positive 36 were male and 29 were female positive (Table 4). Total 65 smear positive cases 21-30 year maximum number of patients (Table 5) and P. Vivax of gametocyte stage were 40 while 11 were schizont stage and P. falciparum of gametocyte were 5 while no schizont stage(Table 6). A total 200 sample 70 were positive and 130 were negative by malaria rapid diagnosis test (Table 7) in which 45 were male and 25 were female (Table 8) and the maximum number of

patient in which 20 were male and 15 were female in 11-20 year age group (Table 9). Out of 200 samples 60 were positive and 140 were negative by QBC (Table10). Total 60 positive sample, in which 35 were male and 25 were female by QBC (Table 11) belong the 21-30 year age group maximum number of patients in which 15 male and 10 female(Table 12). Comparisonof PBS,QBC and malaria RDTs belong to 21-30 year age group 39 PBS, 25 QBC and 35 RDTs maximum number of positive patient (Table 13)(Fig 1).

Table 1: Gender wise distribution

Gender	Patient	Percentage (%)
Male	110	55 %
Female	90	45 %
Total	200	100

Table 2: Age wise distribution of total patients

Age group	Number	Percentage (%)
0-10	10	5%
11-20	30	15%
21-30	70	35%
31-40	11	5.5%
41-50	60	30%
51-60	19	9.5%
Total	200	100

Table 3: Positive and Negative cases by PBS

Sample	Total	Percentage (%)
Positive	65	32.5
Negative	135	67.5
Total	200	100

Table 4: Gender wise distribution of positive and negative cases by PBS

Gender	Male	Female	Total	Percentag
Positive	36	29	65	32.5
Negative	70	65	135	67.5

Table 5: Age and gender wise distribution of positive patients by PBS

Age	Male	Female	Total Positive
0-10	1(1.53%)	1(1.53%)	2(3.61%)
11-20	4(6.15%)	2(3.07%)	6(9.23%)
21-30	20(30.76%)	19(29.23%)	39(60%)
31-40	5(7.69%)	2(3.07%)	7(10.76%)
41-50	3(4.61%)	2(3.07%)	5(7.69%)
51-60	3(4.61%)	3(4.61%)	6(9.23%)
Total	36	29	65

Table 6: Erythrocytic stage & species wise distribution of malaria parasite

Parasite	Schizont Stage	Gametocyte stage	Total
P. vivax	11	40	51
P.falciparum	0	5	5
Total	11	45	65

Table 7: Positive and Negative case by RDT

Sample (n=200)	Total	Percentage (%)
Positive	70	35
Negative	130	65
Total	200	100

Table 8 : Gender wise distribution of positive and negative cases by RDT

Gender	Male	Female	Total
Positive	45(22.5%)	25(12.5%)	70(35%)
Negative	70(35%)	60(30%)	130(65%)
Total	115	85	200

Table 9: Age and Sex wise division of Positive patients by RDT

Age- Group	Male Positive	Female Positive	Total Positive
0-10	5(7.14%)	1(1.42%)	6(8.57%)
11-20	10((14.28%)	5(7.14%)	15(21.42%)
21-30	20(28%)	15(21.42%)	35((50%)
31-40	5(7.14%)	2(2.85%)	7(10%)
41-50	3(4.28%)	1(1.42%)	4(5.71%)
51-60	2(2.85%)	1(1.42%)	3(4.28%)
Total	45	25	70

Table 10: positive and negative cases by QBC

Samples (n=200)	Total	Percentage (%)
Positive	60	30
Negative	140	70
Total	200	

Table 11: Gender wise distribution of positive and negative cases by QBC

Gender	Male	Female	Total	Percentage (%)
Positive	35	25	60	30
Negative	65	75	140	70

Table 12: age and gender wise distribution of positive patients by QBC

Age group	Male positive	Female positive	Total positive
0-10	2(3.34%)	3(5%)	5(8.34%)
11-20	3(5%)	5(8.34%)	8(13.34%)
21-30	15(25%)	10(16.67%)	25(41.67%)
31-40	3(5%)	5(8.34%)	8(13.34%)
41-50	10(16.67)	0(0%)	10(16.67)
51-60	2(3.34%)	2(3.34%)	4(6.67%)
Total	35	25	60

Table 13: Age wise comparison of PBS positive, QBC positive and Malaria antigen card test positive cases.

Age Group	PBS Positive	Malaria antigen card test positive	QBC Positive
0-10	2	6	5
11-20	6	15	8
21-30	39	35	25
31-40	7	7	8
41-50	5	4	10
51-60	6	3	4
Total	65	70	60

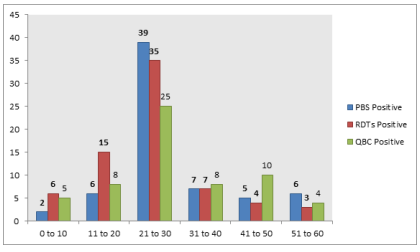

Table 14: Peripheral Blood smear (PBS=65) compare with Malaria Antigen Card Test

Data	Positive blood smear	Negative blood smear	Total
Positive RDTs	60	10	70
Negative RDTs	5	125	130
Total	65	135	200

Table 15: Peripheral Blood Smear(PBS= 65) comparison with Quantitative Buffy Coat Test

Data	Positive Blood smear	Negative Blood smear	Total
Positive QBC	55	5	60
Negative QBC	10	130	140
Total	65	135	200

Calculation:

Sensitivity= (TP/TP+FN)*100
Specificity= (TN/TN+FP)*100

For RDTs

Sensitivity: (60/60+5)*100= 92.30%
Specificity: (135/135+10)*100=93.10%

For QBC

Sensitivity: (55/55+10)*100=91.66%
Specificity: (135/135+5)*100= 96.42%

Discussion:

Out of 200 samples of clinically suspected malaria tested by PBS,QBC and Antigen detection methods,65,60and 70were positive which is similar to K.Lathamani.[9] In their study out of total 100 samples of clinically suspected malaria tested by QBC,PBS and Antigen Card test method, 56,42and 55 were positive. In a similar study done by Chaubey D[10] et al they reported 76 samples positive and 124 negative by PBS out of total 200 samples.

In our study out of total 65 cases 36 were male and 29 females, maximum number of cases were in age group 21-30 year which is similar to Mohammad Gulrez et al[11]. In this study out of total 88 cases 44 were male and 36 were females, maximum number of cases were in age group 1-10 year.

In our study the sensitivity and specificity of antigen card test is 92.3% and 93.1% which is similar to Panigrahi K et al.[12] In this study sensitivity and specificity is 93% and 94.67 % for antigen detection. In this study sensitivity and specificity of QBC were 91.66 % and 96.42 % which is similar to Sonali Bharti et al [13]. In this study the sensitivity and specificity is 94.33% and 97.87 % respectively.

Conclusion:

From our study we conclude that malaria is a serious health issue which requires immediate management so as to administer appropriate treatment of disease and control over the spread.[14] There is a need of an ideal test which is rapid, highly sensitive, cost effective and require less technical expertise to interpret the result. PBS study by microscopy is simple, least expensive but requires time and technical expertise. Malaria antigen card test is rapid and easy to perform but can't focus on the stages of malaria parasite but it detect antigen produce by gametocyte i.e. pLDH can give positive results in infection where only parasite of gametocyte are present but gametocyte are not responsible for the febrile condition. However QBC can be used over peripheral blood smear and RDTs because QBC is rapid, sensitive and does not required trained microscopist as in PBS and nullify the drawback of card test.

As India is a developing country with limited resources and hence PBS which is cost effective, sensitive and also gives accurate species and stages differentiation remains gold standard test.

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