IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 17, Issue 4 Ver. 18 (April. 2018), PP 45-49
www.iosrjournals.org

Comparative Study of Diagnosis of Malaria by Smear Test, Quantitative Buffy Coat (QBC) Method and Rapid Kit Method

Dr.G.Indrani, Dr.K.Sridevi.
Corresponding Author: Dr.K.Sridevi.

***Abstract**: Malaria remains the most important human parasitic infection globally and continues to pose a major public threat in India. The WHO estimates that India accounts for 75% of all malaria cases in South-East Asia[21].Malaria presents a diagnostic challenge to laboratories[9].The present study was aimed at evaluating the sensitivity, specificity, positive predictive value and negative predictive values of QBC, Histidine rich protein-2 and plasmodium lactate dehydrogenase antigen detection test in comparison with peripheral smears in clinically suspected cases of malaria .**Materials and Methods**: Blood samples were collected from 256 clinically suspected cases of malaria of all ages and both sexes between November 2011 to April 2013 SV medical college. Thick and thin blood smears ,quantitative buffy coat examination and one step malaria antigen rapid test using plasmodium lactate dehydrogenase were performed on all the 256 patients. **Results**: Out of 256 cases, 205(80.07%) were positive by blood smear examination by leishman staining. Out of which 172(83.91%) were positive for Plasmodium vivax,33(16.09%) cases were positive of Plasmodium Falciparum. In the present study peripheral smear is taken as gold standard for malaria.199 cases were positive by Quantitative buffy coat examination, of which 167(83.92%) cases were positive for Plasmodium vivax, 32(16.08%) cases were positive for plasmodium falciparum. QBC examination showed sensitivity, specificity of 97.07%, 100% respectively in comparison with blood smear examination.196 cases were positive by malaria antigen Rapid kit method out of which 165(84.18%) cases were positive for plasmodium vivax and 31(15.8%)were positive for plasmodium falciparum. Sensitivity and specificity of PLDH antigen positivity in Rapid Diagnostic Test is 95.61% and 100% respectively in comparison with peripheral smear. **Conclusion:** In the present study we observed though the QBC and Rapid kit method are useful for immediate identification of malarial parasite peripheral smear it still the gold standard*

Date of Submission: 16-04-2018 Date of acceptance: 04-05-2018

I Introduction

Malaria remains the most important human parasitic infection globally and continues to pose a major public threat in India[12].Malaria occurs throughout the tropics causing over 100 million cases and over 1.2 million deaths every year[3,4].In India about 92% of malaria cases and 97% of malaria deaths reported from north-eastern states, chattishgarh, Jharkand, Madhya pradesh, Orissa, Andhra Pradesh ,maharastra, Gujarat, rajasthan, west Bengal and Karnataka[2]. In 2017 in India there were 0.67 million malaria cases, among which 0.44 million cases were due to P.falciparum and annual parasite index was 0.53[22].

Malaria is caused by parasites P.vivax, P.falciparun ,P.malariae and P.ovale. Man develops disease after 10 to 14 days of being bitten by an infective mosquito .There are two types of parasites of human malaria ,plasmodium vivax ,plasmodium falciparum, which are commonly reported from India. Inside the human host, the parasite undergoes a series of changes as a part of complex life cycle. The parasite completes life cycle in liver(pre erythrocytic schizogony) and red blood cells (erythrocytic schizogony).Infection with P.falciparum is the most deadly form of malaria[5,1]

Malaria affects mainly poor, underserved and marginalized populations in remote rural areas which are characterised by inadequate control measures and limited access to health care[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 to clinically diagnose malaria but the treatment has to be started immediately in order to avoid complications. Therefore precise laboratory diagnosis and species identification is essential[4].

In recent years ,numerous quick and new techniques for malaria diagnosis have been developed ,one such being the QBC(quantitative buffy coat)technique[3,6].The other newer technique is rapid diagnostic tests(RDT's)for detection of malaria antigen and enzymes. The antigen detected is histidine rich protein-2(HRP-2)and enzymes detected are plasmodium lactate dehydrogenase (pLDH)and pan-specific aldolase[3].

Materials and methods:

The present study was conducted in the clinical pathology ,S.V.R.R.G. hospital S.V medical college Tirupathi over a period of 1year 6 months between November 2011 and April 2013.A total number of 256 patients with clinical suspicion of malaria were taken for the study. Leishman stained thick and thin blood smears, Quantitative buffy coat examination and one step malaria antigen rapid test(plasmodium lactate dehydrogenase test) were performed on all 256 patients.

Patients presenting with pyrexia and /or atypical presentations, of all age groups and both sex ,attending various outpatient departments and admitted in various wards at S.V.R.R.G hospital,S.V medical college ,Tirupathi are included in the study.

Patients having fever and diagnosed as leptospirosis ,dengue fever, enteric fever ,pneumonia, urinary tract infection and sepsis and Patients who were currently taking antimalarial therapy or who had been treated with antimalarial drugs within the past 2 weeks were excluded from the study .

Informed written consent was obtained from all patients, who were taken for the study. The details of clinical symptoms and signs were recorded and examination of the patient was done to look for presence of splenomegaly and any other complications.

Under aseptic conditions 3 ml of venous blood was collected in ethylene diamine tetra acetic acid (EDTA) tube.1.2mg of EDTA anhydrous salt was added per ml of blood[(10)].

Thick and thin smears were prepared and stained with leishman stain .After staining ,the smears were examined at 100X magnification.100-200 fields ,each containing 20 WBCs were examined before thick smear was reported as negative for malaria. The red blood cells in the tail end of the thin smear were examined under oil immersion for the parasites .The species and stages of the parasite were identified by examination of thin smears

Quantitave buffy coat (QBC)test was done the samples from the study group using malaria tubes of QBC diagnostic INC PHILIPS BURG.

Malaria antigen detection was done using SD bio line one step malaria antigen rapid test for malaria antigen rapid kit test. The kits were all from the same batch and were used before the expiry date indicated by the manufacturer.

II Analysis And Results

All the data was meticulously recorded and analysed. The analysed results were expressed as percentage and proportion with regard to age, sex, clinical diagnosis ,clinical features ,seasonal distribution of malaria positive cases and species of the parasite. Descriptive statistical analysis has been carried out in the present study. Sensitivity ,specificity ,PPV, NPV, accuracy of QBC and rapid kit test were calculated using blood smear examination as the standard reference in the present study.A P value(predictive value)of <0.05 was considered as a significant association between the variables tested.

The age group of the patients in this study ranged from infants to 70 years old. 107(41.8%)out of 256 cases were seen between the age group of 21-40.

Table 1:Age distribution of cases

S.NO	Age(in years)	No of cases(n=256)	Percentage(%)
1	0-10	15	5.9%
2	11-20	47	18.4%
3	21-30	53	20.7%
4	31-40	54	21.1%
5	41-50	44	17.2%
6	51-60	29	11.3%
7	>60	14	5.5%

147(57.4%)out of the 256 cases were males and 109(42.6%) were females.The male to female ratio was 1.35:1.
All the cases presented fever with or without chills and rigors(100%).231 cases (90.2%) presented with body aches,179cases (69.9%) presented with head ache,135 cases(52.7%) presented with nausea and 132 cases(51.6%)presented with anaemia.
Fever with or without chills was the most commonest symptom(100%) followed by bodyaches ,headache, nausea ,anaemia ,abdominal pain splenomegaly ,jaundice ,hepatomegaly and cough with expectoration.
2.3% of patients were presented with cerebral symptoms like altered sensorium ,confusion and irritability,51.6% of the patients had pallor and 2.7% had icterus.7.4% of patients had splenomegaly and 2.73% had hepatomegaly,2.7% of patients had jaundice and 2.3% had cough.
Of the 256 cases tested , 205(80.1%) cases were positive by peripheral smear examination and 51(19.9%) were negative.

Of the 205 positive cases 172(83.91%) were positive for plasmodium vivax 33(16.09%)were positive for plasmodium falciparum.

Table 2:Results of peripheral smear examination

S.NO	Result on smear	No of cases(n=256)	Percentage(%)
1	p.vivax	172	67.2%
2	p.falciparum	33	12.9%
3	negative	51	19.9%

Of the 256 cases tested with QBC,199(77.7%) cases were positive and 57(22.3%) were negative. Of the 199 positive cases ,167(83.92%) were positive for plasmodium vivax,32(16.08%)were positive for plasmodium falciparum.

Table 3:Results of QBC examination

S.No	Result of QBC	No of cases(n=256)	Percentage(%)
1.	p.vivax	167	65.2%
2	p.falciparum	32	12.5%
3	Negative	57	22.3%

Of the 256 cases tested with KIT method(malaria antigen rapid test),196 (76.6%) cases were positive and 60(23.4%)were negative. Of the 196 positive cases,165(84.18%) were positive for plasmodium vivax and 32(15.82%) were positive for plasmodium falciparum.

Table 4:Results of KIT(malaria antigen rapid test)method

S.NO	Result on rapid kit	No of case(n=256)	Percentage(%)
1	p.vivax	165	64.4%
2	p.falciparum	31	12.1%
3	negative	60	23.4%

Out of 256 cases,205(80.1%) were positive in peripheral smear,199(77.7%) were positive by QBC,196(76.5%)were positive by rapid antigen test.

In my study 145 cases(70.73%)cases occurred during may 2012 to November 2012 .peak number of cases 41(20%) were seen during September.

Sensitivity,specificity, positive predictive value, negative predicitive value and diagnostic accuracy of QBC relative to standard criteria, peripheral smear were calculated.

Among 205 cases positive by peripheral smear,199(97.07%) were positive in QBC .All the cases negative by peripheral smear were negative with QBC.

Table 5:comparision of quantitative buffy coat(QBC) examination,in relation to peripheral smear examination

sensitivity	97.07%
specificity	100%
Positive predictive value	100%
Negative predictive value	89.47%
Diagnostic accuracy	97.66%
Cohen's kappa(unweighted)	0.9297

Sensitivity ,specificity ,positive predictive value,negative predictive value and diagnostic accuracy of malarial antigen rapid test(pLDH)relative to standard criteria from peripheral smear were calculated.

Among 205 cases positive by peripheral smear,196(95.06%)were positive in malarial antigen rapid test.All the cases negative by peripheral smear were negative with malarial antigen rapid test.

Table 6:comparision of KIT(malarial antigen rapid test)examination,in relation to peripheral smear examination

sensitivity	95.61%
Specificity	100%
Positive predictive value	100%
Negative predictive value	85%
Diagnostic accuracy	96.48%
Cohen's kappa(unweighted)	0.8967

III Discussion And Conclusion

Malaria is the most important parasitic disease of humans, transmitted in 108 countries containing three billion people and causes nearly one million deaths each year. Malaria has been eliminated from the developed countries like united states however, its prevalence rose in many parts of the tropics including India[7,8].Diagnosis of malaria is still a challenge to laboratories.

The present prospective study was aimed to compare various methods of diagnosis of malaria. Peripheral smear examination quantitative buffy coat method ,and one step malaria antigen rapid test.

Most common age of presentation in the studies like Chandra sekhar et al(n=50),muddaiah m and prakashps(n=314),vipul et al(n=100),sandhya et al (n=500) is >21 years similar to present study(21-40 years).

In all the afore mentioned studies males outnumbered females concordant to the present study.

The most common clinical presentation in the present study is fever(100%)followed by bodyache(90.2%),headache(69.9%).Identical observations are seen in Chandrasekhar et al(n=50)[12]with fever (100%),headache(90.2%),myalgia(69.9%)being the common clinical presentations and vipul et al [20](n=100)and sandhya et al [7](n=500) also showed that fever (84%and 100% respectively)is the most common clinical presentation.

In phommanivong et al[19]too fever (94.4%)was most common clinical presentation.

3028 patients came to clinical pathology lab for peripheral smear examination .out of which 205(7%) cases were positive for malaria in peripheral smear examination.

The slide positivity rate is lower in the present study compared to others since the study was conducted in an area with hot and dry weather commonly.

Plasmodium vivax was the dominant species in the present study similar to the study by muddaiah m and prakash ps[13]majunath et al[3]and saritha yadav et al [15].

The present study had a peak incidence between may to November months similar to singh et al[16] which showed an autumn (October-november) peak for p.falciparum and a summer (april-may) peak for p.vivax.

Peak of malaria cases were recorded in the months of june-july and in October –November coinciding with the rains showing a seasonal pattern by sitalakshmi et al[11].

Sensitivity ,specificity and positive predictive value of QBC in the present study was nearer and better than gay f et al[18] and parija et al[14] but slightly lower sensitivity than oloo aj et al [17] was observed.

Sensitivity ,specificity ,positive predictive value and negative predictive value of pLDh antigen test in the present study was nearer and better than sandhya et al[7]parija et al [14]and saritha yadav et al[15].

IV Conclusion

To conclude though quantitative buffy coat method and Rapid kit method are useful in identifying malarial parasite peripheral smear examination remains gold standard.

References

[1]. Panikar CKJ.Malaria parasites In :Textbook of medical parasitology.6th ed. New Delhi:Jayapee Brothers medical publishers;2007.p.65-95.

[2]. Park K. Park's text book of Preventive and Social medicine.21st edition.Epidemiology of communicable diseases-Malaria,pg.231-243.

[3]. Manjunath P.Salmani,Preeti B.Mindolli,Basavaraj V.Peerapur.Comparative study of peripheral smear,QBC and Antigen detection in Malaria diagnosis.

[4]. PL Bhandari,CV Raghuveer,A Rajeev,PD Bhandari;Comparative study of peripheral blood smear,quantitative buffy coat and modified centrifuged blood smear in malaria diagnosis;Indian journal of Pathology &Microbiology;2008;Volume :51;Issue :1 ;Page :108-112.

[5]. Guruprasad B.A Clinical Study of Hepatorenal and Hematological Profile in Malaria.Tumlur.2011

[6]. Pinto MJW,Rodrigues SR,Desouza R,Verenkar MP.Usefulness of Quantitative Buffy Coat,Blood Parasite Detection System in Diagnosis of Malaria.Indian Journal Of Microbiology.

[7]. Bhat Sandhya K 1#,Sastry Apurba S 1,Nagaraj E.R 2,Sharadadevi Mannur 2,Sastry Anand S.Laboratory diagnosis of malaria by conventional peripheral blood smear examination with Quantitative Buffy Coat(QBC) and Rapid Diagnostic Tests(RDT)-A comparative Study.International Journal of Collaborative Research on Internal Medicine &Public Health.

[8]. Fauci AS,Kasper DL,Longo DL,Braunwald E,Hauser SL ,Jameson JL et al.Malaria.In : Osler W, editor.Harrison's principles of Internal medicine.17th ed.New York:Mc Graw Hill;2008.p.1280-93.

[9]. Moody A.Rapid diagnostic tests for malaria parasites.Clin Microbiol Rev 2002 Jan ;15(1):66-78

[10]. Bain BJ,Lewis SM, Bates haematological techniques .In : Lewis SM,BB, Bates I, editors.Dacie and Lewis practical haematology.10th ed.New York : Churchill Livingstone;2006.p.25-8.

[11]. Wongsrichanalai C,Barcus MJ, Muth S, Sutamihardja A, Wernsdorfer WH.A Review of Malaria Diagnostic Tools : Microscopy and Rapid Diagnostic Test.Am J Trop Med Hyg 2007;77(6):119-27.

[12]. UK Chandrasekhar, KA Sudharshan Murthy, Sunil ;A Study on clinical profile and outcome in falciparum malaria;Calicut Medical Journal 2011 ;9(3):e6.

[13]. Muddaiah M, Prakash PS ; A Study of clinical profile of malaria in a tertiary referral centre in South Canara; J Vector Bourne Dis .2006 Mar;43(1):29-33

[14]. S.C.Parija , Rahul Dhodapkar, Subashini Elongovan , D.R. Chaya; A comparative study of blood smear ,QBC and antigen detection for diagnosis of malaria;Indian Journal of pathology and microbiology -52(2),April-June 2009.

[15]. Sarita Yadav, Madhu Sharma, Aparna and Uma Chaudhary Comparative evaluation of Pan-Malaria Antigen Card Test and blood Smear for Diagnosing Malaria ;Int.J.LifeSc.Bt &Pharma .Res .2012.
[16]. Singh N, Mishra SS, Singh MP, Sharma VP; Seasonality of plasmodium vivax and p.falciparum in tribal villages in central India (1987-1995);Ann Trop Med Parasitol.2000 Mar;94(2);101-12.
[17]. Oloo AJ, Ondijo SO, Genga IO ,Boriga DA, Owaga ML, Ngare D, Gathecha E. Evaluation of the QBC method to detect malaria infections in field surveys. East Afr Med J.1994 May ;71(5):297-9
[18]. Gay F,Traore` B, Zanoni J, Danis M, Gentilini M; Evaluation of the QBC system for the diagnosis of malaria ;Sante .1994 Jul –Aug ;4(4):289-97.
[19]. Viengavaly Phommanivong,Khanti Thongkham, Gopinath Deyer , Jean P Rene Hubert Bareness ; An assessment of early diagnosis and treatment of malaria by village volunteers in the Lao PDR ; Malaria Journal 2010,9:347
[20]. Pankaj P Talviad, T B Javadekar, Bhavan A Selot , Vipul P Chaudhari; Socio Demographic and clinical features of the malaria cases ; National Journal of Community Medicine Vol 3 Issue 1 Jan- March 2012.
[21]. http://www.searo.who.int/india/topic/malaria/en
[22]. nvbdcp.gov.in/malaria3.html

Dr.K.Sridevi.." Comparative Study Of Diagnosis Of Malaria By Smear Test, Quantitative Buffy Coat(QBC)Method And Rapid Kit Method."IOSR Journal of Dental and Medical Sciences (IOSR-JDMS), vol. 17, no. 4, 2018, pp 45-49.