Section: Miscellaneous

Original Research Article

DETECTION AND SPECIES DETERMINATION OF MALARIA PARASITES BY MICROSCOPIC AND NONMICROSCOPIC METHODS.

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ABSTRACT

Background: Malaria is a significant global health challenge, particularly in tropical and subtropical regions. The accurate detection and species determination of malaria parasites is crucial for effective diagnosis and management. Objective: This study aimed to detect and identify malaria parasites using microscopy, serology, and polymerase chain reaction (PCR), and to evaluate their efficacy by comparing them to microscopy, the gold standard.

Material and Methods: Conducted at SCB Medical College, Cuttack, in collaboration with RMRC, Bhubaneswar, this prospective study involved 150 patients from November 2013 to September 2015. Blood samples were examined using thick and thin smears, immunochromatographic tests (ICT), and PCR techniques.

Results: The positivity rates for malaria detection were 73.3% (thick smear), 71.3% (thin smear), 69.3% (ICT), and 80.7% (PCR). PCR showed the highest sensitivity (93.2% for P. falciparum and 75.7% for P. vivax) and specificity (100%) compared to other methods. The study highlighted the advantages and limitations of each diagnostic technique, with PCR being the most sensitive and specific but requiring advanced infrastructure.

Conclusion: Molecular methods like PCR demonstrate superior accuracy in malaria diagnosis, especially in species determination. However, their high cost and complexity make them less feasible in resource-limited settings. ICT offers a viable alternative for rapid and field-level diagnosis despite certain limitations. This study underscores the need for an integrated diagnostic approach to combat malaria effectively.

Key Words: DNA, PCR, ICT.

INTRODUCTION

Malaria is a major public health problem in the tropical and sub-tropical regions, inhabited by nearly 50% of the world's population.[1] Malaria is a protozoan disease caused by Plasmodium spp and is caused by five plasmodium species, namely, Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae and Plasmodium knowlesi. Malaria due to P. falciparum is the most deadly form and causes maximum fatality. Plasmodium species are primarily transmitted by the bite of an infected female anopheles mosquito, but infection can also occur through exposure to infected blood products (transfusion malaria) and by congenital transmission. Malaria continues to pose as a major public health threat in India including the state of Odisha. In 2010, nearly 24% cases and 17% deaths caused by malaria in the country have been reported from Odisha.

Important health consequences of Plasmodium falciparum malarial infections in adults are; cerebral malaria, hypoglycemia, lactic acidosis, non-cardiogenic pulmonary oedema, acute tubular necrosis, anaemia, mild haemolytic jaundice and aspiration pneumonia.

For decades the examination of blood slides by light microscopy has been the cornerstone of malarial diagnosis. This technique is not bereft of flaws, and it requires experience and intensive training on the part of the microscopists. Immunochromatographic methods have taken a lead in this regard, as these are relatively easier to perform but it cannot differentiate amongst all the plasmodia and some of the rapid diagnostic kits are not very useful in the monitoring of response to anti-malarial treatment.[2] Molecular methods have shown a promise in this respect. Analysis of DNA by the polymerase chain reaction(PCR) can be a useful tool for diagnosis of malaria when the results of conventional techniques are negative, especially since PCR allows accurate species identification.

The present study was carried out to Detect and speciate malaria parasites by Microscopy, serology, Polymerase chain reaction and to correlate each other considering microscopy as the gold standard.

MATERIALS AND METHODS

The study is a prospective study conducted in the PG Department of Microbiology, SCB Medical college, Cuttack in collaboration with the Regional Medical Research Centre (RMRC), Bhubaneswar. The study was carried out from Nov 2013 to Sept 2015 extending for a period of two years. The study group comprised of one hundred and fifty patients admitted to different departments of SCB Medical college and Hospital.

Inclusion Criteria: Patients attending the OPD and admitted to inpatient department of Medicine, Paediatrics, Surgery etc having symptoms of malaria such as fever with or without rigor simulating clinical malaria, headache, vomiting alongwith its complications like altered sensorium, convulsions, renal impairment, black water fever, pulmonary oedema, jaundice and diarrhea.

Exclusion Criteria: Patients having fever with symptoms of respiratory tract infections and urinary tract infections.

Sample

From each patient, 4ml of venous blood was collected aseptically. From that 2ml of blood was utilised for preparation of thick and thin smear within 30 minutes of collection and for antigen detection by immunochromatography method. Another 2ml of blood in E.D.T.A. tube was kept at 4°C and transported to RMRC for PCR study.

Method

Thick and thin blood film Preparation where the thick blood films were dehaemoglobinised and then stained with Leishman's stain. Similarly thin smear were also prepared. Immunochromatographic test (ICT): ICT Malaria PF/PV test kits (First Sign-Para view-2) were used as per the manufacturer's instructions PCR analysis for detection of **Plasmodium falciparum**

Isolation and purification of DNA- High molecular weight human genomic DNA was extracted frominfected blood samples by phenol chloroform method with some modification as described by Sambrook & Russel (2001) with slight modification. The concentration and purity of DNA was checked by diluting 5 µl of DNA in 2ml of distilled water and reading the optical density (OD) at 260nm and 280nm in a spectrophotometer.

PCR was carried out in a total volume of 25 µl, containing 4ng parasite DNA, 2 pmol of each primer, 1x Taq polymerase buffer containing 1.5m mol/L MgCl2 and 1 unit of Taq DNA polymerase enzyme. The PCR products were amplified on a programmable thermal cycler. The PCR products were finally separated by electrophoresis on a 2 % agarose gel and visualized by 0.5 µg/ml ethedium bromide staining. The presence of a 918 bp amplicon was viewed with a reference DNA marker under ultra violet light showed the presence of Plasmodium falciparum DNA.

RESULTS

Table 1: Age and sex distribution of cases (n = 150)

Age Group in Years	Male N (%)	Female N (%)	Total N (%)
0-14	09(6)	04(2.7)	13(8.7)
15-25	25(16.7)	13(8.7)	38(25.4)
26-35	35(23.3)	27(18)	62(41.3)
36-45	16(10.7)	06(4)	22(14.7)
46-55	09(6)	05(3.2)	14(9.2)
>56	01(0.7)	00	01(0.7)
Total	95(63.3)	55(36.7)	150(100)

Table 2: Clinical presentation of the cases (n= 150)

Symptoms	Number	Percentage
Fever	150	100
Headache	121	80.7
Chills/rigor	62	41.3
Myalgia	30	20
Vomiting	44	29.3
Altered sensorium	61	40.6
Convulsion	37	24.6
Oliguria/Anuria	6	4

Table 3: Clinical types of malaria and their common symptoms (n=150)

Clinical types of malaria	Number	Percentage	Signs and symptoms
Uncomplicated Malaria	64	42.6	Fever, chills and rigor
Complicated Malaria	86	57.4	Altered sensorium , convulsions, circulatory failure

Out of 150 patients 64(42.6%) presented with uncomplicated malaria and 86 (57.4%) presented with complicated malaria with their common signs and symptoms.

Table 5: Positivity of malaria cases in different diagnostic methods (n=150)

Method	Positive N (%)	Negative N (%)
Thick smear	110(73.3)	40(26.7)
Thin smear	107(71.3)	43(28.7)
ICT	104(69.3)	46(30.7)
PCR	121(80.7)	29(19.3)

Table 6: Percentage vity of plasmodium species with different diagnostic test method positives

Statistical	ICT		PCR	
Test	P.f	P.v	P.f	P.v
Sensitivity	95%	87.50%	93.20%	75.70%
Specificity	91.40%	96.80%	100%	100%
PPV	92.70%	84%	100%	100%
NPV	94.10%	97.60%	91.20%	93.60%

Table 7: Diagnostic methods and their sensitivity and specificity

Method	P.f positive (%)	P.v positive (%)
Thin smear	82(76.6)	25(23.4)
ICT	80(77)	24(23)
PCR	88(72.7)	33(27.3)

Table 8: Shows the percentage positivity of different diagnostic tests with reference to the smear type of Plasmodium vivax species

		THIN SMEAR PV +ve	THIN SMEAR PV -ve	TOTAL	P VALUE
ICT	PV +ve	21[84.0%]	03[2.4%]	150	p<0.001
	PV -ve	04[16%]	122[97.6%]		
PCR	PV +ve	25[100%]	O8[6.4 %]	150	P<o.001
	PV -ve	0	117[93.6%]		
	PV -ve	0	116[92.8%]		

Table 9: Shows the percentage positivity of different diagnostic tests with reference to the smear type of Plasmodium falciparum species

		THIN SMEAR Pf +ve	THIN SMEAR Pf-ve	TOTAL	P VALUE
	Pf +ve	76 [92.7%]	04[5.9%]	150	P<0.001
ICT	Pf -ve	06[7.3%]	64[94.1%]		
	Pf +ve	82[100%]	O6[8.8%]	150	P<0.001
PCR	Pf -ve	0	[62[91.2%]		
	Pf -ve	0	62[91.2%]		

DISCUSSION

Malaria continues to be an important public health problem in India and in many parts of the world. The emerging trend of increasing resistance to antimalarials has affected malaria control programmes world wide as antimalarial chemotherapy continues to be an important component of these programmes. Early diagnosis of malaria not only mitigates the sufferings but also reduces the transmission of the parasite in the community.

In the present study, out of 150 clinically diagnosed malaria cases, 95(63.3%)were males and 55(36.7%) were females. Majority of the patient 62(41.3%) were between the age group of 26-35 years (Table-1). Palatialet al (2013) showed 16-40 years as the common age group in their study, which is comparable to our study.[3]

Our study reported fever as the commonest symptom100% followed by headache 80.7%, rigor and chill 41.3%, altered sensorium 40.6%,nausea and vomiting 29.3% convulsion 24.6%,myalgia 20% and oliguria in 6% cases. (Table -2) Patel et al also reported that fever with or without rigor and body ache is the commonest symptoms and constituted a proportion of 61.11% among all the symptoms which is lower in proportion to our study.[4] Oh M.D. et al. found fever in 100% P.vivax malaria cases Which is similar to our study.[5]

In the present study positive results observed with thick smear, thin smear, ICT, and PCR were 73.3%,

71.3%, 69.3% and 80.7% respectively (Table-5). Pankaj et al (2011) reported 96% detection rate in microscopy and 82% by dipstick method (ICT). Chayani et al detection rate by microscopy and optimal dipstickmethod were 52.5% and 50.8% respectively.[6] There is wide variation in the microscopy result, because microscopic examination is laborious and requires considerable expertise for its interpretation.

The study reported 77% and 72.7% of P.falciparum detection by ICT and PCR respectively. Similarly, the detection of P.vivax in ICT and PCR were 23% and 27.3% respectively (Table-6). Elahi et al.,(2013) reported 73.9% P. falciparum and 26.0% P. vivax from positive cases of microscopy, 74.0% P. falciparum and 25.9% P. vivax by PCR and 74.2% P. falciparum and 25.7% P. vivax byparascreen Method.[7] The definite increase in P. falciparum against low prevelance of P.vivax indicates a high parasite transmission ofP.falciparum in the environment.

In the present study, by comparing immune chromatographic test with microscopy for detection of P. falciparum, 76(92.7%) were found positive by both method, 06(7.3%) positive by microscopy but negative by ICT and 04 (5.9%) negative by microscopy but positive by ICT (Table-7).Dash et al., reported 11.1% of smear negative cases in which ICT was positive.[8] They showed a slightly higher percentage of ICT positive results in smear negative cases than our result.In our study 04(5.9%) cases of smear negative but ICT positive result occurred which might be due to persistence of PfHRP2 antigen in the blood after recovery from acute infection as HRP2 clears very slowly from blood and takes one month forcomplete disappearance after acute infection.

Comparing ICT with peripheral blood smear for detection of P. vivax it was observed that 21(84.0%) were positive by both method and 4(16%) positive by microscopy but ICT negative (false negative) (Table-8). Dash et al., reported false negative results in 3(2.3%) of cases.[8] The microscopic study gives both false positive and false negative results as these errors seem like inherent limitations of microscopy for malaria diagnosis.Similarly, comparing PCR with peripheral blood smear for detection of P. vivax 08(6.4%) cases smear was negative but PCR came positive (Table-10) which is similar to the study of Khan et al., (2013).[9] All the smear positive P.f. and P.v. cases gave positive PCR result.

In our study the sensitivity of PCR to detect P.falciparum and P.vivax was found to be 93.2% & 75.2% and specificity was 100% for both, which is in agree to the study of Singh et al,[10] who reported sensitivity and specificity of PCR was 98% and 100% respectively.The positive predictive value of PCR for P.f & P v in our study was 100% in both which is in accordance with the study of Elahi et al who reported 98.6% and 94.2% respectively.[7]

The present study clearly indicates that molecular test like PCR are highly specific for diagnosis of Plasmodium species whereas they are more or less similar in sensitivity as compared to other diagnostic methods like serological test.

CONCLUSION

In view of the findings presented so far and with the available knowledge, the following conclusions were drawn.

- Malaria is a major global health problem affecting particularly the people living in the tropical and sub-tropical countries.
- One of the most pronounced problem in controlling the morbidity and mortality caused by malaria is limited access to effective diagnosis and treatment in areas where malaria is endemic.
- Microscopic examination of blood smears is the widely used routine method for detection of malaria parasite and remains the gold standard for malaria diagnosis.
- However microscopic examination is laborious and requires considerable expertise for its interpretation, particularly at low levels of parasitaemia.
- Recently, alternative methods, such as immunochromatographic assay, molecular amplification methods like PCR, LAMP have been developed for malaria diagnosis. These methods have some advantages as well as few limitations on one another.
- Among different assays immunochromatographic assays are very simple, rapid stable and give reproducible test results with a threshold detection similar to that obtained by a high quality routine malaria microscopy. It is used in remote areas where suitable infrastructure are not available to meet the requirements for newer sensitive assays.
- But several factors in manufacturing process as well as environmental conditions may affect ICT performance. In addition, exposure of the test kit to high temperature during transport and storage in commonplace in tropical countries can degrade the test kit and bias the result.
- The technique with new modifications offers increased sensitivity and specificity which may be significant in direct identification of drug resistant strains of parasites.
- The molecular method, like PCR is recognized as the most sensitive and specific method of all diagnostic tests for malaria that are currently available.
- The PCR has the disadvantages of being time-consuming and having many steps in the diagnostic procedure. Furthermore, it is expensive because of complex equipment needed to run the PCR assay.
- The Loop mediated isothermal amplification (LAMP) is a molecular method, which in comparison to the PCR is cheaper, simpler and

faster, taking out three disadvantages of the PCR.

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