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Diagnostic Vision and Association with Demographics in Malaria Patients

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A B S T R A C T

Since many years malaria is a scourge of mankind. According to the report, there were 212 million new cases and 429 000 malaria deaths worldwide in 2015. India contributes 70% of malaria cases in the South-East Asia region. This study was conducted as there were only a few studies available in central part of Rajasthan. The main objective is to study the demographic profile of malaria cases and to have a diagnostic vision on them. This was a laboratory based observational, descriptive and retrospective type of study, conducted in Jawahar Lal Nehru Medical College, Ajmer. The data was collected from 1 April2016 to 31 march2017. Statistical analysis was performed with the SPSS, Trial version 23 for Windows statistical software package (SPSS inc., Chicago, il, USA) and Primer. A total 27,455patients were tested by rapid diagnostic method and 3810 slides were examined. Out of which 502 and 79 were positive for malaria, respectively. A total of 530 malaria positive cases, 59.32% were male and 40.67% were female. Maximum number were found in age group 21 to 30 years and <10 years which was 23.77% and 22.08%. A total number of cases were highest in September (22.64%). In RDT out of 502 positive cases, *P. vivax* and *P. falciparum* were 54.91% and 39.81% were as in slide test. Out of 79 cases 79.74%, 17.72% and 2.53% were *P. vivax, P. falciparum and* both respectively. Early diagnosis and control activities well ahead of the monsoon season should target more to the communities with lower socio-economic status and the young age groups.

Introduction

In India malaria was discovered by Sir, Ronald Ross in 1897. A century have passed, after its discovery, still malaria continues to be one of the leading public health problem in India (Estimation of True Malaria Burden in India).

Malaria is a mosquito-borne parasitic disease caused by several species of the *Plasmodium* parasite. Genus Plasmodium has five species, such as *P. falciparum, P. vivax, P. ovale, P. malariae* are human malaria species and *P. knowlesi* has been recorded recently causing malaria among monkeys in certain areas of South-East Asia. *P. falciparum* and *P. Vivax* malaria pose the greatest public health challenge. (Dharmendra Prasad Singh *et al.*, 2016). Human malaria is transmitted by 60 species of female *Anopheles* mosquito. Out of 45 species of Anopheles mosquito in India, only few are regarded as vectors which are *An. culicifacies,*

An. fluviatilis, An. stephensi, An. minimus, An. philippinensis, An. sundaicus, etc. (Paniker's textbook of medical parasitology).

Malaria life cycle passes its life in 2 host; definitive host is female Anopheles mosquito and intermediate host is Man. There are mainly 2 phases of development in human host.one is inside the liver which includes pre erythrocytic schizogony in which no clinical and no pathogenicity occur and hypnozoite stage which is the cause of relapse. Other is in RBC that is erythrocytic phase which includes erythrocytic schizogony that causes malarial paroxysm and gametogony that is an infect mosquito (Chatterjee *et al.*, 2009).

The clinical manifestation in malaria involves a series of febrile paroxysms like cold stage, hot stage and sweating stage, followed by splenomegaly and anaemia (Chatterjee *et al.*, 2009)

Malaria typically manifests as a febrile illness that can be fatal if unrecognized, especially in young children (Alireza Salimi Khorashad *et al.*, 2014).

Microscopy is the reference/gold standard for the laboratory diagnosis of malaria parasite but its turnaround time is much more than that of RDT and it requires adequate training. RDTs are alternative diagnostic methods because they are quick and easy to carry out (Ruby Naz *et al.*, 2016).

According to the report, there were 212 million new cases and 429 000 malaria deaths worldwide in 2015. India contributes 70% of malaria cases in the South-East Asia region. This study was done as there are few studies available in central part of Rajasthan.

The Objective was to study the demographic profile of malaria cases and to have a diagnostic vision on them.

Materials and Methods

This study was carried out at Department of Microbiology, Pathology Laboratory Jawahar Lal Nehru Medical College, Ajmer, India, over a period of one year from 1 April 2016 to 31 march2017.

Study design

Observational, descriptive study.

Study type

Retrospective type of study.

Statistical test

Statistical analysis was performed with the SPSS, Trial version 23 for Windows statistical software package (SPSS inc., Chicago, il, USA) and Primer.

The Categorical data were presented as numbers (percent) and were compared among groups using Chi square test.

Inclusion criteria

A total 31,265 suspected malaria cases were subjected to smear microscopy or rapid diagnostic tests. (Rapid card Test=27455; Slide test=3810).

Exclusion criteria

Patients with other positive laboratory test results i.e. for typhoid fever and dengue fever.

Five ml blood (3ml in case of children) was collected through venipuncture by expert technician in full aseptic conditions. Peripheral thick and thin blood smears were prepared on same slide and stained with Leishmann stain as per standard laboratory protocol.

Microscopy (done in pathology lab)

Leishmann stained blood films were examined under oil immersion fields. Stained slides were considered positive when at least one parasite was found, and negative if no parasite is found in 200 oil fields. Thin smear blood film was examined for species identification.

Rapid diagnostic tests (done in microbiology lab)

Commercially available ParaHIT Total ver.1.0 was used for detection malarial antigens such as HRP-2 antigen and Pan specific aldolase antigen.

Principle

Immuno-chromatography, in which nitrocellulose membrane is coated with anti-HRP II antibody which is specific for *P. falciparum* and anti-aldolase antibody which detects the presence of any of Plasmodium species.

Procedure

Take 8mL of sample with micropipette window A of test device Add 4 drops (200mL) of reaction buffer to window B of test device read the test at the end of 25 minutes.

Results and Discussion

A total 31,265 suspected malaria cases were subjected to smear microscopy or rapid diagnostic tests. (Rapid card Test=27455; Slide test=3810). Out of which 530 cases were positive for malaria.

No significant difference was observed in gender according to age group. Most of the cases were observed in 21 to years of age group as compared to extreme of age group. Males were more in all age group as compared to females in all age groups (Table 1).

There were 503 test positive for malaria through rapid diagnostic test, out of Pan (which is considered as *Plasmodium vivax* as there is no species existing in this area other than *Plasmodium falciparum*) were maximum that was 291(57.96%) was found to be maximum followed by *Plasmodium falciparum* and mix. In case of slide test out of 79 positive cases again results were same *Plasmodium vivax* was maximum that were 63(79.74%) (Fig. 1).

The maximum number of cases was more in August, September and October month (Fig. 2).

Specificity and PPV of rapid diagnostic test were 99.60%, 80.60% and sensitivity and NPV were 86.2%, 99% (Table 2).

Malaria is an important parasitic infection affecting large populations and continues to remain a serious public health problem in India.

In our study most of the cases were observed in 21 to30 years of age group followed by age group 1 to 10 years which was 23.78% and 22.08% respectively. Males were more in all age group as compared to females in all age groups.

Our study was similar with a study done in U.P by (Dharmendra Prasad Singh *et al.*, (2016)) with a slight higher range affected in the above age groups that was 47.59% and 23.30% respectively and the number of males dominated over the females. Our study also coincided with a study done in Navi Mumbai by (Gurjeet Singh *et al.*, (2015)). However, in a study conducted by EE Ayougu *et al.*, (2016) in Nigeria 53 (34.40%) were males while 101 (65.50%) were females.

Table.1 Baseline characteristics of the study population

No of cases	**530**	
Age Groups	**No**	%
<1Year	9	1.69
1TO 10	117	22.08
11T O20	94	17.73
21 TO30	126	23.78
31 TO40	72	13.58
41 TO 50	64	12.08
51TO60	20	3.78
61 TO 70	16	3.01
>70	12	2.27

	F		**M**		**Total**
	No	**%**	**No**	**%**	
<1yEAR	3	33.33	6	66.67	9
1TO 10	47	40.17	70	59.83	117
11T O20	29	30.85	65	69.15	94
21 TO30	48	38.10	78	61.90	126
31 TO40	22	30.56	50	69.44	72
41 TO 50	27	42.19	37	57.81	64
51TO60	6	30.00	14	70.00	20
61 TO 70	7	43.75	9	56.25	16
>70	6	50.00	6	50.00	12
	195	36.79	335	63.21	530

Chi-square = 5.775 with 8 degrees of freedom; P = 0.672 NS

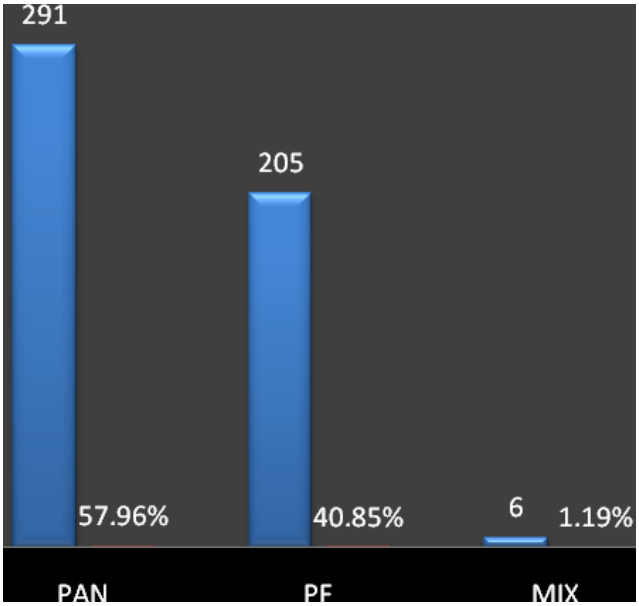
Table.2 Evaluation of rapid malaria kit with microscopy

	Microscopy positive	**Microscopy negative**	**TOTAL**	**SN**	**SP**	**PPV**	**NPV**	**ACCURACY**	**P VALUE**
Rapid Reactive	50	12	62	86.21%	98.25%	80.65%	99.73%	99.34%	P <0.001S
Rapid Nonreactive	8	2930	2938						
	58	2942	3000						

SN: sensitivity Table number: 2); SP: specificity PPV; positive predictive value, NPV; negative predictive value;
Chi-square = 2053.858 with 1 degree of freedom.

Fig.1 Distribution of the positive cases according to test

Rapid Diagnostic Test (502)



Slide examination (79)

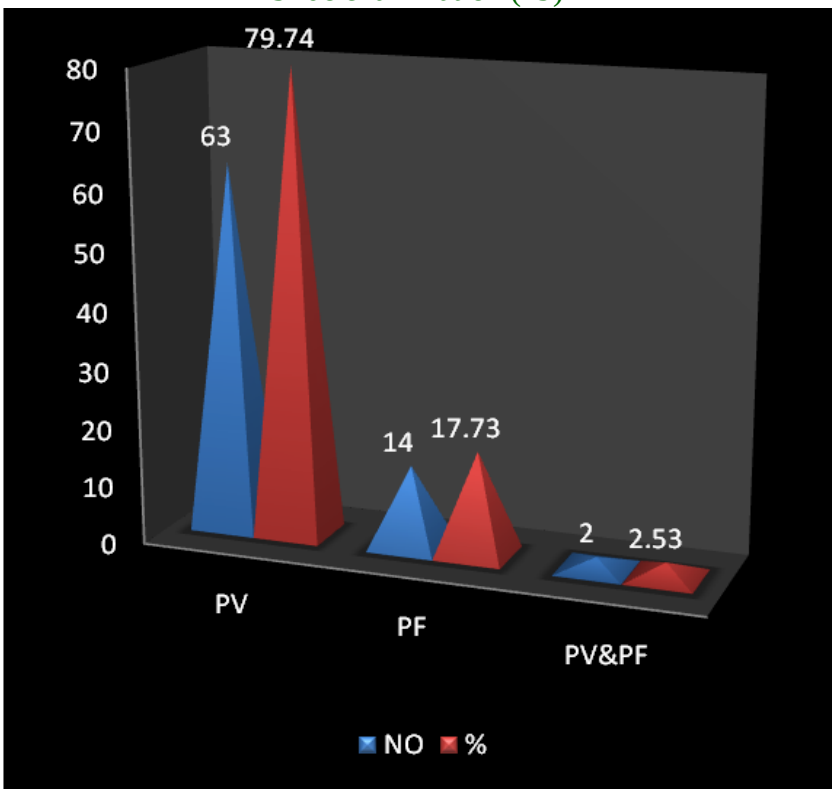
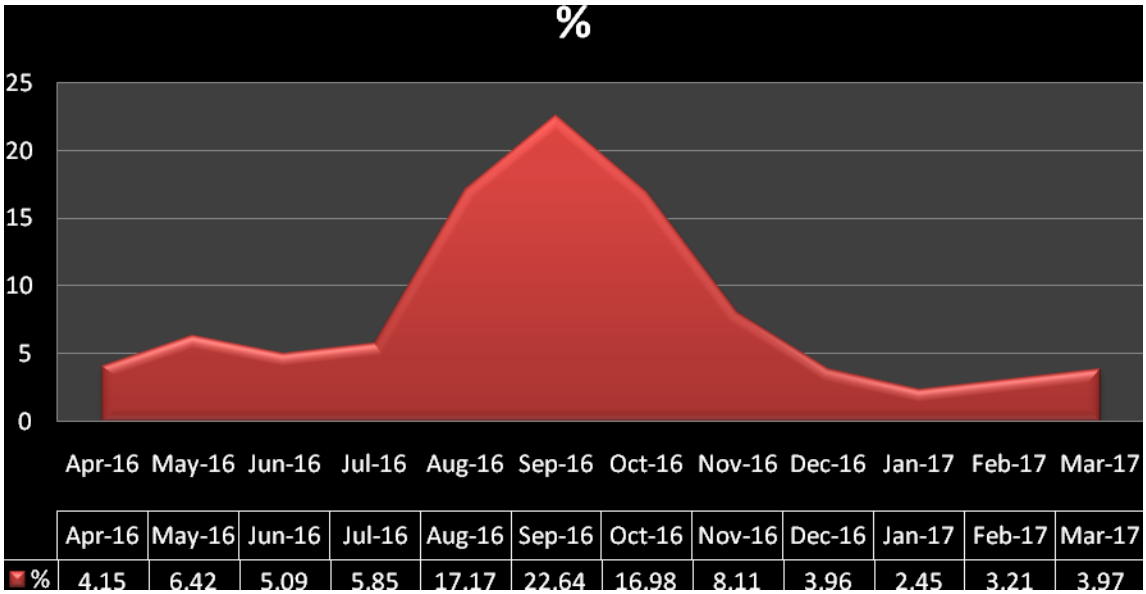


Fig.2 Seasonal distribution of positive cases



Also a study done by Nmadu *et al.,* (2012) 64% cases were in the age group 1 to 10 years in Nigeria. This can be due to differences socio-economic conditions of patients, knowledge about healthcare and public health practices.

In the present study, *Plasmodium falciparum*, *Plasmodium vivax* and mixed species infection was 56.70%, 41.80%&01.50% respectively. Our study results were close to study done by Dharmendra Prasad Singh *et al.,* (2016) whose result were 67%, 32% & 1% respectively. However, Karlekar *et al.,* (2012) from Gadchiroli reported *Plasmodium vivax* 33.80% and *Plasmodium falciparum* 66.60%. This can be due to differences in geographical variation of the spp.

In this study cases were more in August, September and October month. Our results were similar to study done by Pankaj Gangwal *et al.,* (2016) and Gurjeet Singh *et al.,* (2015)). The high prevalence of malaria in this period could be due to collection of water in rainy season and mosquito breeding.

In our study Specificity and PPV of rapid diagnostic test were 99.60%, 80.60% and sensitivity and NPV were 86.2%, 99%, our findings were consistent with the study done by Ruby Naz *et al.,* (2016) which was 93.60%, 87.20% and 91.30%, 95.70% respectively. So, it is a good diagnostic and a screening test.

The severe illness and outbreaks emphasizes the importance of rapid diagnosis to decrease the related complications and thereby combating significant mortality and morbidity.

Our study revealed that the district still have the potential to suffer Malaria outbreak, if necessary interventions are not done for prevention and control of the disease. Early diagnosis and control activities well ahead of the pre-monsoon and monsoon season should target more to the communities with lower

socio-economic status and the young age groups mostly males. Lower prevalence of malaria after 30 years of age could be attributed to development of immunity (resistance) due to clinical or subclinical exposures.

Rapid diagnostic test for diagnosis of malaria is as reliable and accurate as microscopy. Rapid diagnostic test use should be considered as more cost effective in the areas characterized by high-moderate intensity malaria transmission and in situations where health services are inadequate or absent.

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