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"Insights into Malaria Diagnosis: Correlating Microscopy and Rapid Detection test along with Clinical Biomarkers in *Plasmodium vivax* Malaria at North India

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ABSTRACT

Background: The incidence of *Plasmodium vivax (P.vivax)* cases that cause malaria has increased in the Indian subcontinent. Malaria has emerged as a significant research issue due to the severity of the disease and changes in laboratory test results brought on by *P. vivax*.

Aim and Objective: With the objectives of evaluating the pattern of change in lab parameters in illness and its role in comprehensive management of the treatment response, the current study aims to demonstrate the association between malaria and altered lab parameters and to compare the diagnostic techniques among the patients from Uttar Pradesh.

Material and Methods: This was a retrospective study carried out in the Department of Microbiology of the central laboratory at Sharda Hospital in Greater Noida over a period of ten-months (April 2023–January 2024). A rapid malaria antigen test (RMAT) and a peripheral blood smear examination (PBS) were both used to confirm malaria. Automation was used for the hematological and liver function tests.

Results: In the present study 46 out of 790 patients tested positive for malaria, out of which 42 had *P. vivax* infection and 4 had *Plasmodium falciparum (P. falciparum)*. Male patients from urban households made up the majority of the patients. When compared to the non-infected patients, the confirmed positive patients exhibit a significant derangement in hemoglobin levels, hemotocrit (Hct), platelet counts, aspartate transaminase (AST), total bilirubin, and direct bilirubin. The sensitivity and specificity of RMAT are 44.88% and 98.86%, respectively. The positive predictive value (PPV) and negative predictive value (NPV) were found to be 95.6% and 97.7%, respectively.

Conclusion: For patients suffering from malaria in endemic areas, basic blood testing can provide a diagnostic clue.

Keywords- Malaria, *Plasmodium vivax*, Laboratory parameters, Urban malaria, sensitivity, specificity

INTRODUCTION

Malaria is a serious global public health issue that causes substantial morbidity and mortality, particularly in tropical and subtropical countries (1). It is caused by the protozoan parasite of the genus plasmodium, which attacks and damage the red blood cells. There are 5 species (*Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium ovale* and *Plasmodium knowlesi*) that cause human malaria, of which *P. falciparum* have the high morbidity and mortality

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(1,2). According to the latest world malaria report of 2022, there were 249 million cases and 60,800 deaths due to malaria. With geographical differences in prevalence and incidence rate, *P. falciparum* and *P. vivax* are the main pathogenic species in India accountable for 1.4% of total malarial cases in the world (3). It is transmitted to humans via the bite of a female Anopheles mosquito. In India, the primary vector for transmitting the disease is *Anopheles stephensi.* It was formerly believed that *P. vivax* causes a benign tertian malaria, but in recent years this tendency has shifted, with life-threatening symptoms comparable to those of *P. falciparum* infections (4). Numerous cases of severe illness progression and mortality have been recorded, including problems in various organs that were not before observed. It is unclear what pathophysiological mechanisms underlie the various clinical manifestations of *P. vivax* malaria (5,6). Malaria infection causes hematological alterations such as anemia, thrombocytopenia, and reduced hematocrit. The sporozoite form of the parasite can damage liver cells, causing organ congestion and cellular inflammation. These anomalies in hepatocytes can cause the leakage of parenchymal and membranous liver enzymes like aspartate aminotransferase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) into the bloodstream, resulting in their increased levels in the serum of malaria-infected patients (2). The significant changes in these indicators between the acute and convalescent clinical phases may prove beneficial for detecting the progression of the disease and the early response of the patient to the therapy (5). One-third of all malaria cases found in India are caused by *P. vivax*, which is more common in urban settlements (4). According to studies, demographic characteristics, climate, socioeconomic status, population movement, and proximity to mosquito breeding sites are all risk factors for *P. vivax* infection (7). The diagnostic approach to the disease needs to be accurate and quick. Malaria detection mostly relies on microscopy and RMATs, which have a significant impact on its control. Light microscopy is considered the gold standard for diagnosis. It requires a high level of expertise and skilled personnel to examine peripheral blood thick and thin smears. But it has its own limitations; without a skilled person or adequate lab infrastructure, it cannot be performed. While we have a RMAT kit that follows the principle of lateral flow assay, it has now been employed as a point of care diagnosis without the requirement of a skilled person or lab infrastructure, but it does pose the doubt of giving false positive results. In order to achieve the best diagnostic approach, both technologies should be combined, considering the present constraints (8,9).

MATERIAL AND METHODOLOGY

This retrospective study was carried out in the microbiology department of the central laboratory at Sharda Hospital in Greater Noida over a period of ten-months (April 2023–January 2024).

Inclusion criteria- Samples of suspected symptomatic patients with positive malaria test.

Exclusion criteria- Samples of suspected symptomatic patients with negative malaria test.
The diagnosis and confirmation of malaria was made based on the detection of malaria parasites by conventional thick and thin peripheral blood films, stained with Giemsa stain, and RMAT.
The RMAT were based on the detection of specific Plasmodium antigen, lactate dehydrogenase. The CareStart™ malaria parasite lactate dehydrogenase/histidine-rich protein 2 (pLDH/HRP2) combo (Pf/Pv) test kit was used.
Hematological analysis- An automated hematology analyzer (SYSMEX.XN-1000) was used.
Liver function test assay- A fully automated biochemistry analyzer, the Vitros 5600, was used.

Statistical analysis- The data was collected and entered into MS Excel, and the statistical analysis was performed using the SPSS software. A p value of less than 0.05 was judged significant. The data is mostly composed of numerical figures and percentages.

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RESULTS

A total of 790 suspected samples were received in the microbiology lab of Sharda Hospital, 46 of which tested positive. The total positivity rate was calculated to be 5.8% (46/790). The predominant Plasmodium species detected was *P. vivax*, infecting 91.3% (42/46) of all the suspected cases, followed by *P. falciparum* at 8.6% (4/46).
As depicted in Figure 1, males represent 63% (29/46) of the positive 46 samples, whereas females represent 37% (17/46) of the total positive samples

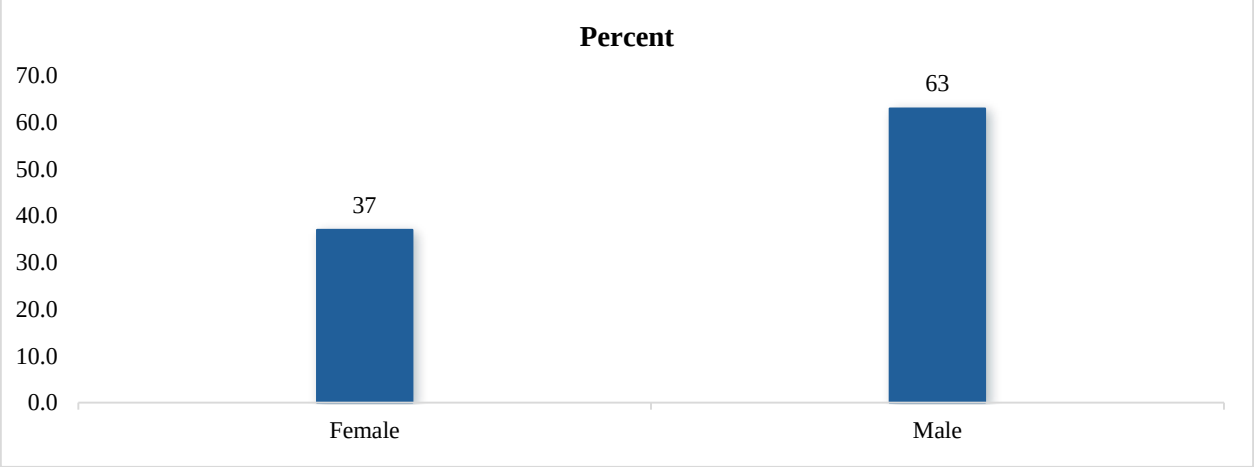

Figure 1: Gender wise distribution of positive isolates (n=46)

Figure 2 shows the age-wise distribution of the positive samples. The majority of infected patients were under the age of ten (35%,16 /46), followed by those between the ages of 10 to 20 (17%,8 /46), and those aged 31 to 40 (17%, 8/46), age group between 21 to 30, 41 to 50 and above fifty were 15% (7/46), 11% (5/46) and 4% (2/46) respectively.

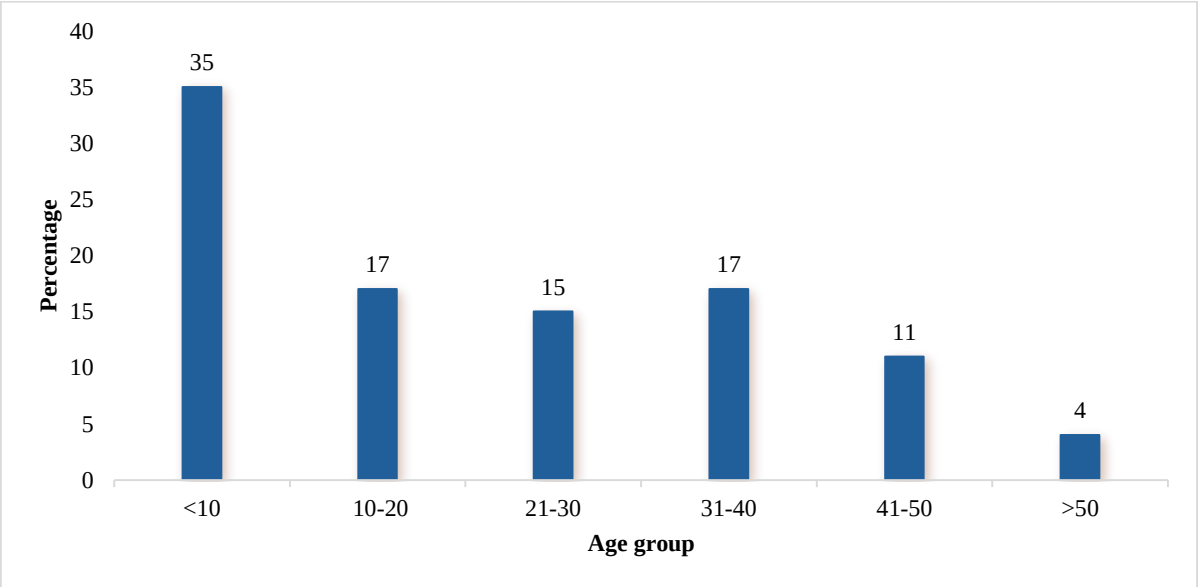

Figure 2: Age wise distribution of positive isolates (n=46)

Figure 3 depicts the seasonal pattern of the disease. The maximum cases were recorded in the month of September (37%, 17/46), followed by the month of June (22%, 10/46), August (17%, 8/46), July (15%, 7/46), May and October (4%, 2/46). However, no samples were received in the months of November, December and January.

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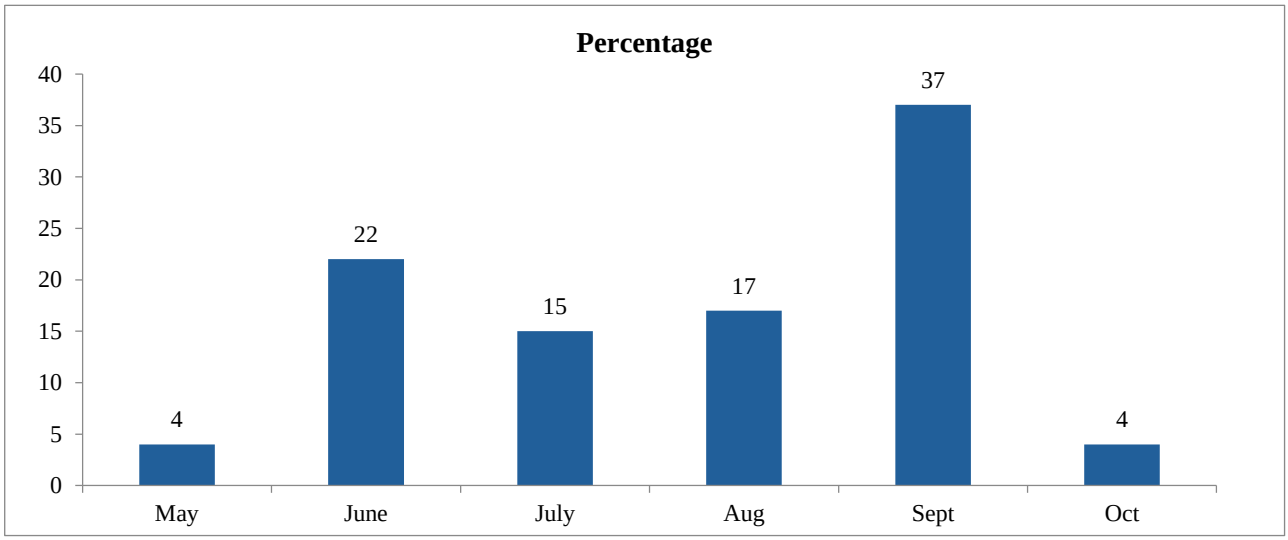

Figure 3: Seasonal pattern of Malaria disease

Figure 4 shows the housing style of the confirmed positive isolates. The majority of the patients belong to urban households, that is, 54% (25/46), followed by patients belonging to rural households, which are 46% (21/46).

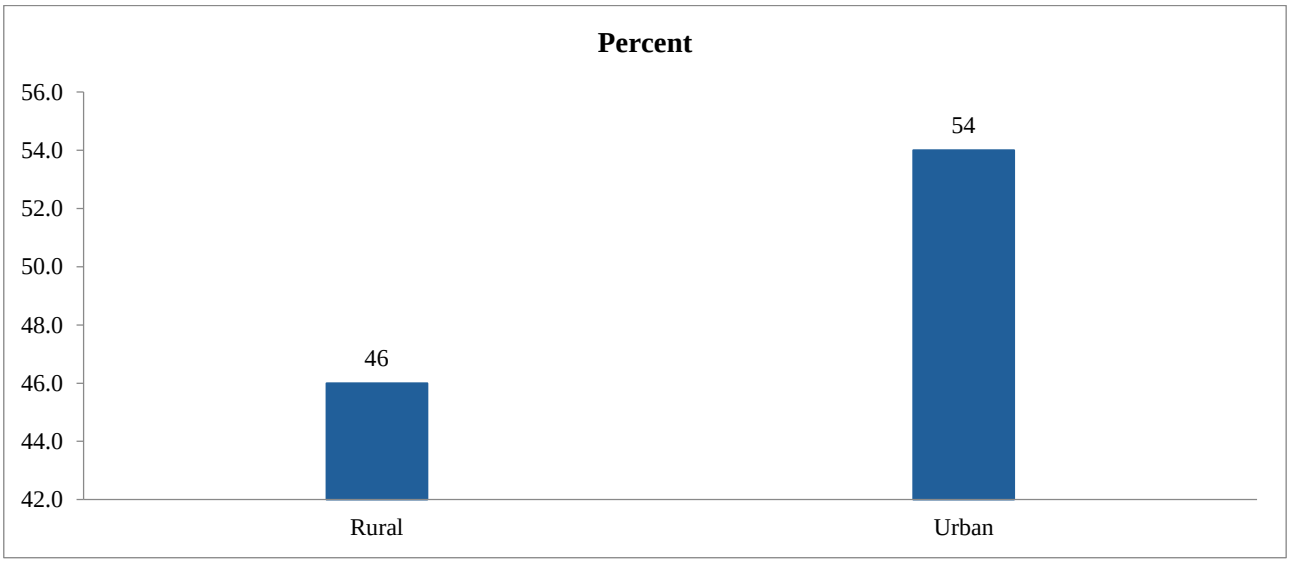

Figure 4: Housing style of the isolates

As shown in figure 5, out of 46 positive malaria cases, twenty-three samples were positive by RMAT alone, one came out to be positive by PBS microscopy alone, and twenty-three samples were positive by both RMAT and PBS microscopy.

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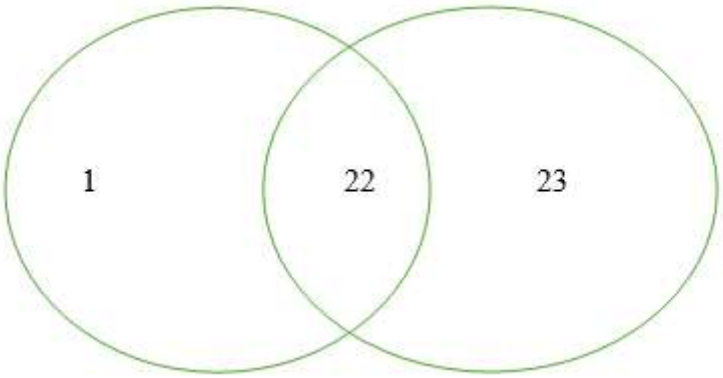

Figure 5: Venn diagram showing distribution of positive cases by different RDT and PBS microscopy

Table 1 shows the comparison of hematological parameters between infected and non-infected subjects. Hemoglobin, hct, and platelets show significantly decreased levels with p values of 0.001, 0.006, and 0.000, respectively, while lymphocytes and neutrophils did not show any significant deranged levels, which are 0.135 and 0.074, respectively.

Table 1: Comparison of hematological markers

Parameters	Infected subjects	Non-infected subjects	p- value
Hb (g/dl)			
Severe (<5)	6	0	0.001
Moderate (5-9)	14	2	
Mild (9-12)	19	16	
>12	7	726	
Hematocrit (%)			
Low	33	8	0.006
Normal	12	735	
High	1	1	
Lymphocytes			
Low	25	17	0.135
Normal	12	713	
High	9	14	
Neutrophils			
Neutropenia	8	16	0.074
Normal	21	709	
Neutrophilia	17	19	
Platelets (cmm3)			
<50,000	26	0	0.000
50,000-1,50000	15	44	
1,50,000-400000	5	700	

Table 2 depicts the comparison of various liver function biomarkers between infected and non-infected subjects. AST, total bilirubin, and direct bilirubin show significant deranged levels with p values of 0.016, 0.001, and 0.019, respectively, while ALT and ALP did not show any significant deranged levels, which are 0.144 and 0.131, respectively.

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Table-2 Comparison of Liver functions biomarkers

Parameters	Infected subjects	Non-infected subjects	p- value
AST (0-32 U/L)			
Raised	31	7	
Normal	15	737	0.016
ALT (0-50 U/L)			
Raised	32	4	
Normal	14	740	0.144
ALP (38-126 U/L)			
Raised	29	9	
Normal	17	735	0.131
TB (0.2-1.3 mg/dl)			
High	21	1	
Normal	25	743	
Low	8	0	0.001
DB (0-0.4 mg/dl)			
High	26	08	0.019
Normal	20	736	

Table 3 shows the percentage of case positivity of the diagnostic methods used to confirm malaria.

Table 3- Positivity rate by RMAT and PBS microscopy methods

Sample	PBS Microscopy	RMAT
Positive	23	45
Negative	767	745
Total	790	790
Positive percentage	2.9%	5.6%

Table 4 shows the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), which are 48.88%, 99.86%, 85.6%, and 97.7%, respectively, by taking PBS microscopy as a gold standard.

Table 4- Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of RMAT.

Statistical analysis	Total detected (n=46)
PBS microscopy confirmed	1
RMAT confirmed	23
Both PBS microscopy and RMAT confirmed	22
Sensitivity	48.88%
Specificity	99.86%
PPV	95.6%
NPV	97.7%

DISCUSSION

The burden of malaria in India is complex because of the highly variable geographical and epidemiological profiles and transmission factors. In the present study, malaria was detected in only 46 out of 790 suspected cases with the prevalence rate of 5.8% which was comparable to the findings recorded by Bal M et al with prevalence rate of 7.8% in North India (10).The majority of Plasmodium species found in our study were *P. vivax* (91.3%, n = 44/46) followed*P. falciparum* (8.6%) which was detected in only 4 suspected cases. This was consistent with the

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observations by Karoli et al. and Jairajpuri ZS et al., reporting *P. vivax as* more common than *P. falciparum* in the region, with rates of 56.5% and 84.8%, respectively (11,12). This study indicated a high number of male malaria-confirmed cases as compared to the females, suggesting higher infection rates among males in the study area. This finding was comparable with a study reported by Jairajpuri ZS et al and by Hassen J et al (12,13). The probable reason could be because of greater exposure in the males. We also noted that most of the cases were recorded in the age group of 0–10 years, followed by 31–40 years, which is similar to a study done by Kochar DK et al. (14), which reported a high affinity for *P. vivax* infection in children <5 years of age. The most likely explanation for this could be the lack of acquired immunity in small children (15).Malaria transmission was recorded from April 2023 to January 2024. A large number of malaria-infected patients were observed just following the rainy season. The highest prevalence was recorded in the month of September. This was consistent with the findings of Tigu F et al. and Nigussie TZ et al. (16,17), which indicated that the month of September was the peak for the incidence of parasitic infection throughout the autumn season. This is due to damp places and waterlogging leading to stagnation of water, which produces an ideal habitat for mosquito breeding. Furthermore, crops like maize are most cultivated during this time of the year, and pollen grains produced by it may serve as a food supply for mosquito larvae to complete their life cycle (16). Rapid and unplanned urbanisation leads to the creation of urban slums and a high inflow of population density with poor hygiene and sanitary conditions, promoting breeding grounds for vector mosquitoes with the potential to transmit disease. Acknowledging the same notion, our study ascertained 54.3% of urban malaria cases, compared to 45.7% of rural malaria cases. This similar finding is seen in the study done by Lendongo Wombo JB et al. (18), where they reported a significant ubiquity of severe malaria in urban households.On comparing the clinical biomarkers within infected and non-infected groups we found, majority of infected subjects had hemoglobin levels below 12 g/dl (92.8%, n=39/42) out of which 41.3% (n=19/46) had mild, 30.4% (n=14/46) had moderate and 13% (n=6/46) had severe anaemia. Also, the infected group was significantly associated with decreased hematocrit (78.5%, n=33/42). As the parasite matures, it causes the loss of infected red blood cells, but many unaffected red blood cells are damaged because of membrane modifications, antibody stimulation, and the hyperactivity of reticuloendothelial cells in the spleen, leading to the suppression of erythropoiesis (5). Hematocrit, haemoglobin concentration, and thrombocyte count show statistically significant deranged p values of (<0.006), (p<0.001) and (p<0.000), respectively, which is in concordance to the study conducted by Al-Salahy et al. and SR Parveen et al. (2,19). Globally, much research is ongoing on the accurate mechanism of thrombocytopenia in malaria. Many studies have contemplated the theory of oxidative stress, splenic pooling, immunoglobulin G (IgG)-mediated platelet destruction, and structural abnormalities of platelets brought on by Plasmodium (20). Total bilirubin (0.001), direct bilirubin (0,019) and aspartate transaminase (0.016) were also found significantly deranged in confirmed cases compared to the malaria negative group. These findingswere in agreement with studies done by Alves-Junior ER et al and Bhattacharjee et al (5,21). With the rise in parasite density in the liver, an increase in levels of bilirubin and leakage of liver enzymes is seen. Hyperbilirubinemia can be caused by the destruction of hepatocytes or the hemolysis of both parasitized and nonparasitized red blood cells (2). In recent years, RMATs have been viewed as an excellent alternative approach for diagnosing malaria. It is recommended to employ RMAT in conjunction with microscopy. In our study, the sensitivity and specificity of RMAT were 48.88% and 99.86%, respectively, with a positive predictive value and a negative predictive value of 95.6% and 97.7%, which is similar to the findings of the study conducted by Gupta P et al., where they found the sensitivity and specificity of RMAT to be 91.8% and 93.8%, respectively. The positive predictive value and negative predictive value were found to be 97.8% and 98.9%, respectively (9).

To achieve the elimination goal, the country must properly and consistently implement policies and practices which are used for the intervention of this neglected species. Poor diagnostic performance, low parasite density, the presence of gametocytes especially in asymptomatic infections, and the coexistence of different malarial species in the country hinder the progress towards elimination. So

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to achieve the elimination, sensitive diagnostic tools with good performance in detecting low parasitaemia infections and accurately identifying malarial species are necessary [22].

CONCLUSION

In India, where malaria is a major cause of febrile illness, the findings of this study delve into the complex terrain of malaria diagnosis, with a special emphasis on *Plasmodium vivax* malaria in North India. Our study focuses on the role of the factors like socio-economic status and seasonal pattern along with laboratory parameters to detect the prevalence of malaria in western Uttar Pradesh, North India. The disease burden in our hospital was 5.8%, and most of the patients were infected by *P. vivax* (91.3%), followed by *P. falciparum* (8.6%). Males from rural households under the age of ten years were primarily affected by malaria. This study also confirms the statistically significant correlation between the parameters like anaemia, hematocrit, thrombocytopenia and elevated liver enzymes with *P.vivax* malaria, thus, providing the diagnostic clue .
On the other hand, insufficient positive samples jeopardised any significant correlation among *Plasmodium falciparum* malaria. As single center and small sample size were the limitations of the presesnt study further large-scale multicentric studies with the more clinico-epidemeological parameters involving large populations would be need of the hour.

Declarations:
Conflicts of interest: There is no any conflict of interest associated with this study
Consent to participate: We have consent to participate.
Consent for publication: We have consent for the publication of this paper.
Authors' contributions: All the authors equally contributed the work

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