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Evaluation of Hematological Parameters with Biochemical Correlation in Malaria a Cross-Sectional Study

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ABSTRACT

Malaria is a major health problem in India leading to anemia, thrombocytopenia and leukopenia and also damages the kidneys and liver if left untreated. Evaluation of hematological parameters and biochemical correlation play an important role in malaria. The aims were to analyze the distribution of demographic data, clinical and pathological characteristics, hematological parameters and biochemical tests in malaria. From January to December 2022, a retrospective observational study using a cross-sectional approach was conducted at Rama medical college and Research Centre. The study included 47 malaria cases and 47 healthy controls. The study examined symptoms and frequency of changes in hematological and biochemical parameters. Laboratory values were recorded and analyzed for associations. Data were evaluated using SPSS version 26 (trial version), compared the study groups with the control group using standard t-tests to identify statistically significant differences. The Anova test was used to analyze biochemical tests. Findings with a p-value below 0.05 were deemed to be of statistical significance. P. vivax was the most commonly observed species, at 43 (91%), while P. falciparum accounted for 4 (9%) of cases. Out of 47 cases, 31 were males., the majority of the cases belonged to people 13-30 years of age. The most frequent hematological abnormalities were normochromic normocytic anemia and thrombocytopenia. The study shows significant mean values for hemoglobin (HB), hematocrit (HCT), red cell distribution width (RDW), total leukocyte count (TLC) and platelet count (PC), but not for mean cell volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration. The correlation of hematological parameters with parasitemia grades was statistically insignificant. Hyperbilirubinemia was the most common. Blood urea was elevated in more cases than creatinine. Plasmodium vivax was the most common malaria species in our region, predominantly affecting males of young to middle age. Early diagnosis and management could be facilitated by monitoring HB, HCT, RDW, TLC and platelet values. Kidney and liver function assessments could prevent organ damage.

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Key Words
Malaria, anemia, thrombocytopenia

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INTRODUCTION

Malaria stands as a significant global health issue[1]. The latest data from the World Health Organization (WHO) in 2023 indicate that there were approximately 249 million instances of malaria across 85 countries where the disease is prevalent in 2022, a slight increase from 244 million cases in the previous year. Notably, India and Indonesia were responsible for 94% of malaria- related fatalities within the South-East Asia Region[2]. This disease is caused by Plasmodium protozoa, which are transmitted by female Anopheles mosquitoes when they bite. Other less common transmission methods include blood transfusions, needle sharing and from mother to child during pregnancy[2]. Plasmodium thrives in warm, humid conditions and is particularly prevalent in India during the months from July to November, with states such as Gujarat reporting high numbers of Plasmodium vivax (P. vivax) and Plasmodium falciparum (P. falciparum) cases[3-4]. The incubation period for P. vivax typically ranges from 8-17 days, while for P. falciparum, it ranges from 9-14 days[5]. Infection with malarial parasites leads to alterations in blood composition, resulting in a range of blood disorders including anemia, thrombocytopenia, changes in white blood cell counts and abnormal platelet function and count[6].

The purpose and objectives of this study were to examine the distribution of demographic data, observe the clinical and pathological characteristics, assess and evaluate hematological parameters and investigate and correlate biochemical tests with malaria.

MATERIALS AND METHODS

From January to December 2022, a retrospective observational study using a cross-sectional approach was conducted at the Central Hematology Laboratory within the Pathology Department of a tertiary care center.

Ethical Statement: The study was approved by the ethical committee and was conducted according to the Declaration of Ethical Committee of Rama Medical college and Research Centre Hapur.

Research Participants: The study included 47 individuals previously diagnosed with malaria and 47 healthy controls for comparative purposes.

Criteria for inclusion: The study welcomed cases that exhibited positive smears for at least one species of malaria parasite. It considers both patients receiving inpatient care and those treated as outpatients.

Criteria for Exclusion: This study did not include patients whose malaria diagnosis was solely determined through rapid antigen detection tests.

Study Methods and Data Collection: Patient information, such as age, sex, clinical manifestations, symptoms and initial diagnoses, were gathered from inpatient medical records and outpatient laboratory systems. Hematological analyses involved the collection of 2-4 mL of venous blood using EDTA vacutainers. The blood was processed and analyzed within 2-4 hours of collection using the Sysmex KX-21 automated 3-part hematology analyzer. The recorded data included hemoglobin (HB), hematocrit (HCT), total leukocyte count (TLC), differential leukocyte count (DLC) and platelet count (PC).

Blood smear analysis revealed thin and thick smears. A thick blood smear was considered negative if no parasites were detected across 100 oil-immersion fields. Parasite density in thick smears was quantified using a plus system scale to indicate parasitemia levels in positive cases: +(1-10 parasites per 100 thick-film fields), ++(11-100 parasites per 100 thick-film fields), +++(1-10 parasites per thick-film field) and ++++ (>10 parasites per thick-film field)[7].

Peripheral blood smears (PBS) were meticulously reviewed under various magnifications for red blood cell (RBC) morphology, TLC, DLC, platelet sufficiency, and malarial parasite type. Serum samples from the patients were analyzed to determine liver function indicators using Xl-640, a fully automated biochemistry analyzer. Serum bilirubin (both direct and indirect), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and serum proteins were measured. Renal function was assessed using a fully automated Erba XL-640 biochemistry analyzer and serum creatinine and blood urea levels were recorded.

Data Analysis: Demographic and hematological data, including variables such as HB, RBC count, HCT, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), PC, mean platelet volume (MPV) and platelet distribution width (PDW), were evaluated using SPSS version 26 (trial version). This analysis compared the study groups with the control group using standard t-tests to identify statistically significant differences. Additionally, a biochemical examination was conducted on serum bilirubin, serum creatinine and blood urea levels in malaria patients using the ANOVA test of analysis. Findings with a p-value below 0.05 were deemed to be of statistical significance.

RESULTS AND DISCUSSIONS

Throughout the study period, the Central Hematology Laboratory of the Pathology Department conducted 56,774 complete blood counts. Of the 2,469 individuals tested for malaria, 47 were confirmed to have the infection through peripheral blood smear examinations

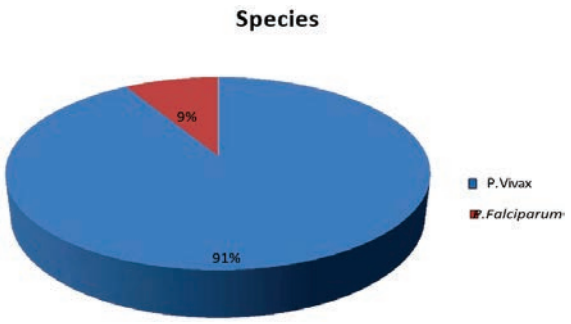

Fig. 1: Species Distribution of the Present Study

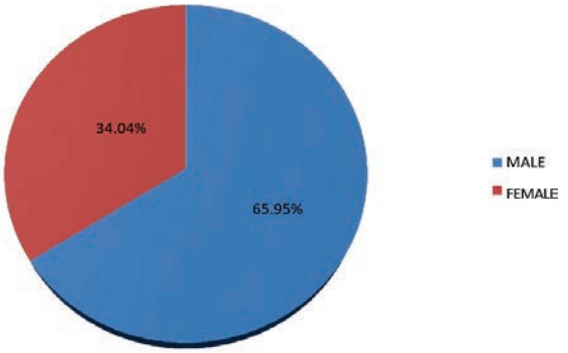

Fig. 2: Sex Distribution of the Present Study

and were enrolled in the current study. Among these patients, 14 were admitted as inpatients and 33 were treated as outpatients. Most cases were documented from July to October. Among the 47 confirmed cases, P. vivax was the predominant species, with 43 cases accounting for 91% of the total. P. Falciparum was identified in 4 of the cases, representing 9% of the Sample (Fig 1).

The study observed a predominance of male participants, constituting 31 individuals (approximately 65.95%), whereas female participants numbered 16(34.04%) (Fig 2).

As shown in Figure 3, the participants ranged in age from 13-60-years-old. The mean age of participants was 32 years. The majority of the patients were in the 16-30 years age group, followed by those 46-60 years.

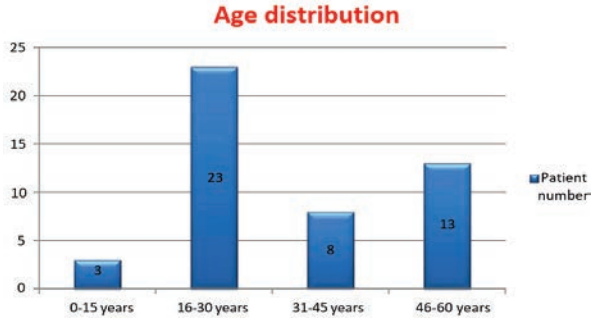

Fig. 3: Age Distribution of the Present Study

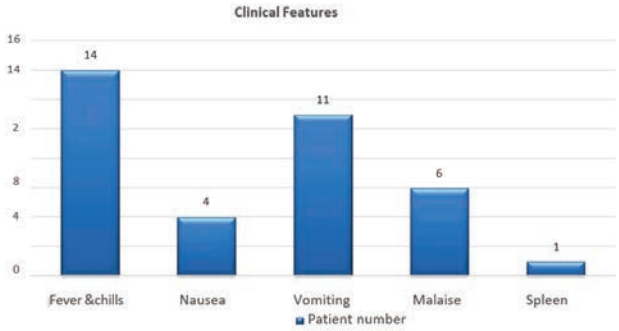

Fig. 4: Symptoms of the Study Participants of the Present Study.

(Fig 3). Within the age group, a significant majority (91.4%) of the subjects were diagnosed with P. Vivax infection, whereas the rest (8.1%) were diagnosed with P. falciparum infection. Of the 31 males, the majority were infected with P. Vivax (n=28, 90.3%), while the remainder were infected with P. Falciparum (n=3, 9.67%). Among the 16 females, the majority were infected with P. Vivax (n=15, 93.75%), while only one individual (6.25%) was infected with P. falciparum.

Symptoms were examined in 14 of 47 indoor patients. Symptoms included fever, chills, nausea, vomiting and malaise. The most prevalent symptoms were fever and chills, followed by vomiting. Splenomegaly was found in one indoor patient (Fig 4).

Anemia affected 14 individuals (29.78%), with an equal distribution between mild and moderate anemia. This investigation found no cases of severe anemia. In the case of anemia, females outnumbered males by nine (Table 1).

RBC count, HCT, RDW, TLC and PC were significantly associated ($p < 0.05$). Most patients (65.95%) had normal TLC, while the majority (76.69%) had thrombocytopenia (Table 2).

Most individuals had a normocytic normochromic morphology (Table 3).

In the LFT analysis, serum total bilirubin was elevated in 78.37% of the patients, but AST, ALT, ALP and serum protein levels were generally normal (Table 4).

*RBC: Red Blood Cell., HCT: Hematocrit., MCV: Mean Corpuscular Volume., MCH: Mean Corpuscular Hemoglobin., MCHC: Mean Corpuscular Hemoglobin., RDW- Red Cell Distribution Width., TLC: Total Leucocyte Count., PC: Platelet Count.

HB, RBC count, HCT, MCV, TLC, and PC were not significantly associated with parasitemia grade ($p>0.05$, Table 5).

P. vivax malaria was more prevalent than P. falciparum malaria (91.48% vs. 8.51%, respectively). No cases involved other species. Similar to the current

Table 1:Number of Males and Females with Mild, Moderate and Severe Anemia

Anemia(Hemoglobin)	Mild anemia (9.0-11.0mg/dl)	Moderate anemia(6.1-8.9mg/dl)	Severe anemia(<6.0mg/dl)	Total
Male	2	3	0	5/31
Female	5	4	0	9/16
Total	7(14.89%)	7(14.89%)	0	14/47

Table 2: Frequency Distribution of RBC Count, RBC Indices, RDW, TLC and PC in the Present Study

Parameter(unit)	Value	(n =47.100%)	Mean±SD(*IQR)	P-value
RBC count(Millions/cmm)	<4.1	18(38.29%) 21		
	4.1-5.1>5.1	(44.68%) 8(17.02%)	4.22±8.50	P<0.0001
HCT(%)	<38	29(61.70%)	34.99±7.99	P<0.0001
	38-51	18(38.29%)		
	>51	0		
MCV(fl)	<80	11(23.40%)	87.2(9.7)*	P=0.865
	80-96	23(48.93%)		
	>96	3(6.38%)		
MCH(pg)	<26.5	13(27.65%)	28.6(3.9)*	P=0.548
	26.5-33.5	32(68.08%)		
	>33.5	2(4.25%)		
MCHC(g/dl)	<31.5	8(17.02%)	32.8(2.2)*	P=0.186
	31.5-35.5	38(80.85%)		
	>35.5	1(2.12%)		
RDW(%)	11.7-14.4	34(72.34%)	15.83±2.09	P<0.0001
	>14.4	13(27.65%)		
TLC(/cmm)	<4000	16(34.04%)	5128.29±2016.18	P<0.0001
	4000-11000	31(65.95%)		
	>11000	0		
PC(lacs)	<1.5	36(76.69%)	97648.93+79289.6	P<0.0001
	1.5-4.5	11(23.40%)		

Table 3:RBC Morphology on Peripheral Blood Smears in the Study Participants

RBC Morphology			
Species	**Normocytic/Normochromic RBC n (%)**	**Normocytic/Hypochromic RBC n (%)**	**Macrocytic RBC n (%)**
P.falciparum	04(8.5)	-	-
P.vivax	30(63.8)	11(23.4)	02(4.3)
Total(47)	34(72.3)	11(23.4)	02(4.3)

Table 4: Analysis of Biochemical Parameters in the Study Participants

Parameters	Value	n(%)
Total Billirubin	0.2-1 >1	8(21.62%) 29(78.37%)
Direct Billirubin	0-0.2 >0.2	2(5.45%) 35(94.59%)
Indirect Billirubin	<0.3 0.3-0.7 >0.7	1(2.702%) 10(27.02%) 26(70.27%)
ALT	0-32 >32	22(49.45%) 15(40.54%)
AST	0-31 >31	22(49.45%) 15(40.54%)
ALP	38-94 >94	27(72.97%) 10(27.02%)
Serum Total Protein	<6.0 6.0-8.3 >8.3	16(43.24%) 21(56.75%) 0

Table 5: Correlation of Hematological Parameters with Grading of Parasitemia

Parasitemia Grade	HB Mean (SD)	RBC Count Means (SD)	HCT Means (SD)	MCV Means (SD)	TLC Means (SD)	PC Means (SD)
1	11.46(2.73)	4.16(0.63)	34.48(7.42)	82.4(11.72)	5200(1978.36)	55840(48281.34)
2	11.47(2.45)	3.97(0.82)	34.73(6.98)	83.68(17.25)	4780(2030.70)	111834.78(87256.31)
3	12.67(2.46)	4.54(0.69)	39.07(6.54)	85.97(4.83)	5305(1993.34)	87350(75502.47)
4	9.6	4.64	32.9	70.9	4100	45000
P-Value	0.695	0.519	0.688	0.857	0.916	0.491

investigation, Akhter[8], Ullah[9] from Pakistan and Shah[10] from Gujarat all identified P. vivax as the primary species causing malaria, accounting for 84%, 56.6% and 77%, respectively. In contrast, P. falciparum was the most common species reported by Sakzabre[11] from Ghana (87.3%) and Karlkekar[12] from Maharashtra. The WHO reports that 53% of the P. vivax burden is in Southeast Asia, with India accounting for 47%. Malaria caused by P. vivax and P. falciparum is approximately 51:49[13-14]. This study found that more males were infected (65.95% vs. 34.04%), which is consistent with the findings of Akhter[18](64%), UllahS[19] (52.9%), Shah[18](65%) and Awok N and Arota[15] (68%). Males may engage in more outside activities, which could explain the relative prevalence of men. In a study conducted by Sakzabre[20] in Ghana, they discovered that females were more affected (69.07%), which contradicts the current data. The gender-based variations in the current study's findings could be attributed to variables in size, population density, climate and geographic location, as well as disparities in the health counseling practices of men and women. Overall, men are more likely to take medication than women. When a woman feels ill, she visits a doctor.

The majority of the patients were between 16 and 30 years of age. Sakzabre[21] found similar results with the majority of patients aged 18-44 years. Fourteen

patients (29.78%) with anemia were classified as mild (n=7, 14.89%) or moderate (n=7,14.89%). The difference in Hb levels was statistically significant (P>0.0001). The majority of anemic patients (n=13, 92.8%) had P. vivax and the majority of these patients (n=9, 64.2%) were female. P. falciparum was primarily seen in male patients (n=2). These results disagreed with those of a study by Shah[22] that indicated 53.1% of cases had anemia. The fact that the majority of research participants were male may be the reason why this study found fewer cases of anemia. Although the study's highest number of P. vivax species in anemia was comparable to that of Shah[23] (P. vivax 76.9%). Seasonal or regional variances can occur. The majority of patients (n=34, 72.34%) had normocytic normochromic RBC shapes, regardless of the species. All P. falciparum malaria patients showed normocytic normochromic RBC, whereas 63.8% of P. Vivax-positive individuals had similar morphology, followed by normocytic and hypochromic RBCs (23.4%). The macrocytic RBC morphology was the least common (4.3%). These findings were consistent with those of Shah[24], who reported normocytic normochromic RBCs in 46.6% of cases, with 21.2% of patients having microcytic hypochromic RBCs. In a rural investigation, the inclusion of previously iron deficient patients may have revealed the occurrence of microcytic hypochromic anemia. Malaria usually causes normocytic normochromic anemia. Severe hemolysis can reveal macrocytic RBCs.

CONCLUSION

In contrast to the findings of Antwi Bafour[16], who concluded that TLC and MCH had a significant correlation with the degree of parasitemia at 95% and 99% confidence levels, respectively and that TLC and PC showed statistically significant relationships, our attempt to correlate hematological parameters with parasitemia grades was found to be statistically insignificant (p>0.05, Table 5). RBC counts were normal in the majority of patients (n=21), with significantly decreased and increased counts in 18 and 8 patients, respectively (P>0.0001).

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