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Original research article

To study the effects of the parasite load on the haematological parameters

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Abstract

Background: Malaria is one of the infectious diseases that poses one of the greatest loads in terms of morbidity and mortality, and as a result, India has a significant problem with the disease. In spite of the early success of the malaria eradication campaign in the 50s and 60s, there was an upsurge of cases to 6.74 million in 1976, which declined to 2.1 million in 1984. In 1984, the number of people infected with malaria reached its lowest point since the programme began. Since that time, it has stabilised at this level.

Methods: This prospective study was carried out between June 2021 and May, 2022 at department of pathology, Kurnool Medical College, Kurnool, a tertiary care facility serving the surrounding areas. The 200 malaria cases with smear positive results found at the Malaria Laboratory were all chosen, and haematological alterations were examined. This investigation on the haematological characteristics of malaria was carried out at a hospital connected to a medical school. Numerous haematological parameters were examined in each of the 100 malaria cases that tested positive on a smear.

Result and Discussion: Both falciparum and vivax infections can cause a variety of haematological abnormalities, most frequently normocytic normochromic anaemia and thrombocytopenia. Generally speaking, parasite load and the degree of anaemia and thrombocytopenia are correlated. In a patient with a febrile condition, meticulous examination for the malarial parasite is necessary in order to observe thrombocytopenia. White blood cell changes are less pronounced, and the outcomes of different investigations vary. Leucopenia, leucocytosis, and rarely the presence of aberrant lymphocytes are among the reported modifications.

Conclusion: The goal of the current study was to track haematological alterations in 200 hospitalised malaria subjects with positive smear tests. There were 51% cases of vivax, 48% of falciparum, and one mixed illness. In the current study, 58% of the participants were men, compared to 69% in earlier studies with identical findings. Leucocytosis was observed in 10% of the cases in the current investigation. 18% of the patients in the current study had leucopenia. In the current study, 81% of the malaria cases had thrombocytopenia. Utilizing statistical analysis, the current study found a correlation between the level of anaemia and the parasite load.

Keywords: Malaria, plasmodium, parasite, hematological, normochromic anaemia

Introduction

The word "malaria" originates from Italian, where it was believed that the disease was caused by the foul air that is common near marshy areas. More than 2.4 billion people around the world are at risk of contracting malaria, and more than 100 countries around the world are considered malarious [1-3]. Malaria kills between 1.1 and 2.7 million people annually, the majority of whom are children under the age of five. Anemia, thrombocytopenia, leucocytosis, leucopenia, mild to moderate atypical lymphocytosis, monocytosis, eosinophilia, and neutrophilia are some of the haematological changes that have been reported to accompany malaria. Platelet abnormalities can be qualitative as well as quantitative. Thrombocytopenia is a common occurrence in acute malaria and it is observed in vivax and falciform infections [4-6].

In the course of a malaria infection, haematological alterations can occur, including anaemia, thrombocytopenia, and either leukocytosis or leucopenia. These changes are well known. The extent of malarial endemicity, background hemoglobinopathy, dietary state, demographic characteristics, and malaria immunity are all factors that can influence how these changes manifest. Since more than twenty years ago, the World Health Organization (WHO) has included hyperparasitemia as one of the criteria for determining whether or not a patient has severe falciparum malaria [7-10]. Previous research has demonstrated that the number of parasites present in an area is directly proportional to the severity of the patient's malaria infection. There is also a correlation between the severity of parasitemia and mortality.

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Patients who have the highest levels of parasite density also have the highest rates of patient mortality. In addition, anaemia is a significant complication that can arise from a high parasitemia caused by a Plasmodium falciparum infection. In addition, anaemia can be caused by the increased hemolysis of parasitized red blood cells in patients who have malaria. In addition, thrombocytopenia was observed in the vast majority of patients who were diagnosed with malaria. Platelets were discovered to be much lower when there was a high level of parasitemia. falciparum parasite burdens [11-14]. This relationship was found to be significant. The purpose of this research was to demonstrate the effect that infections with Plasmodium falciparum and Plasmodium vivax, as well as varying parasite densities, had on the blood cell parameters of patients suffering from malaria. Patients who were infected with malaria had their haematological parameters examined. These parameters included red blood cells, white blood cells, platelets, leukocytes, haemoglobin level (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and red cell distribution width (RDW) [15-17].

Materials and Methods

Source of data: A prospective study will be carried out at the hospital, which is a tertiary care facility serving surrounding districts and is linked to department of pathology, Kurnool Medical College between June 2021 and May, 2022. All two hundred and fifty cases of malaria that tested positive for smears at the Malaria Laboratory were chosen at random and examined for haematological alterations.

Collection of Data: In accordance with the prescribed procedure, a comprehensive history was obtained from the patient, including information about their age, gender, the nature and duration of their sickness, any previous blood transfusions, and any previous treatments for malaria. The findings of the clinical examination were noted. Before beginning treatment with anti-malarial medication in any of these instances, a blood sample was collected for a haematological analysis. Venous blood was collected in EDTA Vacutainers as well as 3.8% sodium citrate vacutainers (for ESR estimation). Haemoglobin (Hb), Haematocrit (HCT), RBC indices like MCV (mean corpuscular volume), MCH (mean corpuscular haemoglobin), MCHC (mean corpuscular haemoglobin concentration), total leucocyte count, absolute leucocyte counts Using a fresh blood sample, thin blood smears were produced and stained with leishman stain before being examined for blood picture, differential leucocyte count, species identification, and calculation of parasitaemia. The percentage of parasitaemia in thin blood smears was determined by counting the number of infected RBCs in relation to the total number of RBCs in the sample, which was set at 200.On a thick smear a rough estimate of parasite concentration count was obtained by observing average number of parasites per thick blood field as follows

1. 1-10 Parasites per 100 thick film fields
2. 11-100 Parasites per 100 thick film fields
3. 1-10 Parasites per thick film field
4. More than 10 Parasites per thick film field Biochemical values were available in some cases.
5. Normal ranges for haemogram findings were taken from J.U.

Observations

This investigation on the haematological profile of malaria was carried out during the months of between June 2021 and May, 2022, Kurnool Medical College, Kurnool, that is connected to the medical college. Numerous haematological parameters were investigated on each of the one hundred smear-positive malaria cases that were collected. There were approximately the same number of vivax and falciparum infections among the total of 200 patients that were examined. In addition to this, one patient was found to have a mixed infection.

Table 1: Total number of cases with different Infections

Type of Parasites	Number of Patients	Percentage (%)
P. vivax	102	51%
P. falciparum	96	48%
Mixed	02	01%
Total	200	100%

There were almost equally as many vivax (51%) and falciparum (48%) infections. This is a manifestation of the general trend of rising falciparum cases. Two peaks were visible in the distribution of cases during the study period, the first in October–November and the second in May–July. The majority of occurrences occurred during these times.

Table 2: Age Distribution of Malaria Patients

Age Group (Years)	Number of Patients (n=200)	Percentage (%)
< 5	24	12
6-14	22	11

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15-30	52	26
31-45	48	24
>45	54	27

According to the table, young adults between the ages of 15 and 45 accounted for the majority of cases (50%) while minors (23%) also had a sizable number of instances. There were people of various ages present; the youngest was a female kid under 2 months old with P. vivax illness, and the oldest was an adult female with P. falciparum infection.

Table 3: Sex distribution of cases

Malaria species	Male (n=109)	Female (n=91)
P. Falciparum	48	50
P. Vivax	59	41
Mixed	02	-
Total (n=200)	109	91

While falciparum cases were equal in both sexes, vivax infections were found more in males.

Table 4: Showing duration of illness in Malaria Patients

Duration of Illness (in days)	Number of Patients	Percentage (%)
< 7	156	78
8 -14	26	13
15 – 21	10	05
> 21	08	04
Total	200	100

Most of the patients presented with illness duration of less than 7 days (78%). Only 4% of the patients presented with duration more than 3 weeks.

Table 5: Presenting Symptoms in different Malaria Infections

Symptoms	P. falciparum (n=96)	P. vivax (n=102)	Mixed (n=02)	Total	Percentage (%)
Fever	96	102	02	200	100
Chills and Rigor	78	60	02	138	69
Nausea and Vomiting	40	08	02	50	25
Myalgia	10	16	02	28	14
Pain Abdomen	12	04	-	16	8
Diarrhea	06	02	-	08	4
Cough	08	12	-	20	10
Breathlessness	04	04	-	08	4
Headache	16	16	02	34	17
Altered sensorium	06	-	02	08	4

Table 6 demonstrates that fever was present in every instance; the second most prevalent symptom, chills and rigour, was present in 69% of patients. There were 50 cases of nausea and vomiting, of which the majority (40 cases) were caused by falciparum malaria. Three falciparum infections and one mixed infection had altered sensoriums, but none of the vivax infections did.

Table 6: Clinical Signs in Malaria Infections

Signs	P. falciparum (n=96)	P. vivax (n=102)	Mixed (n=1)	Total (n=200)	Percentage (%)
Pallor	60	70	-	130	65
Jaundice	14	20	02	36	18
oedema	08	04	-	12	06
lymphadenopathy	02	02	-	04	02
hepatomegaly	10	02	-	12	06
splenomegaly	08	12	-	20	20
hepatosplenomegaly	26	28	-	54	27
Abdominal tenderness	10	04	02	16	08
Basal crepitens	04	-	-	04	02
tachycardia	02	-	-	02	01
Neck rigidity	08		02	10	05
Extensor plantar reflex	06	-	02	08	04

The most frequent clinical indicator was pallor. In 27% of cases, there is hepatosplenomegaly, and the prevalence of falciparum and vivax malaria is about equal. Neck rigidity, tachycardia, and basal crepitens

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are more frequent in falciparum malaria.

Table 7: Complications of Malaria

	P. falciparum (n=96)	P. Vivax (n=102)	Mixed (n=02)	Percentage (%)
Cerebral Malaria	08	-	02	10
Severe Anaemia (<5g/ dl)	14	08	-	22
Acute Renal Failure	04	02	-	06
Respiratory Complications	04	-	-	04

Severe anaemia (5 gm %) was the most frequent consequence, occurring in 22 instances while falciparum infections occurred in 7 cases. There were 10 cases of cerebral malaria, of which 8 were caused by falciparum and 2 by mixed infection. Pulmonary problems and acute renal failure were both observed in 6 and 4 patients, respectively.

Biochemical Parameters

Table 8: Total Bilirubin

Total Bilirubin (mg/dl)	P. vivax (n=06)	P. falciparum (n=10)	Total (n=16)
<3	04	02	06
>3	02	08	10

Table 9: Blood urea

Blood urea	P. vivax (n=16)	P. falciparum (n=24)	Total (n=40)
Normal	08	08	16
Elevated	08	16	24

Table 10: Serum Creatinine

Serum Creatinine	P. vivax (n=14)	P. falciparum (n=26)	Total (n=40)
Normal	02	10	12
Elevated	12	16	24

In 12 cases, there were high blood urea levels, and in 24 cases, there were elevated serum creatinine levels. Infections with falciparum were more likely to have altered biochemical markers.

Red Cell Parameters

Table 11: Hb (Haemoglobin) Concentration

Hb (gm/dl)	P. vivax (n=102)	P. falciparum (n=96)	Mixed (n=02)	Percentage (%)
< 5	08	14	-	11
5 – 8	40	32	02	37
8 – 11	39	44	-	40
> 11	18	06	-	12

As shown in the table, majority of the patients had either mild (40%) or moderate degree (37%) of anaemia. Hb Concentration <5 gm% was seen in 11% of the cases; mainly in falciparum infection.

Table 12: RBC Count

RBC Count (million/µl)	P. vivax (n=102)	P. falciparum (n=96)	Mixed (n=02)	Percentage (%)
< 3	44	58	-	51
3 – 4	40	28	02	35
4 – 5	16	10	-	13
> 5	01	-	-	01

Severe RBC count reduction was more common in falciparum malaria (29%) as compared to vivax (22%).

Table 13: HCT (Haematocrit) (%)

HCT %	P. vivax (n=102)	P. falciparum (n=96)	Percentage (%)
< 20	22	26	24
20 - 35	70	68	69
> 35	10	02	06

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Haematocrit values of less than 20 were seen more commonly in falciparum infection. One case of mixed infection showed haematocrit of 20.5%.

Table 14: Peripheral Blood Picture in Malaria species

Blood Picture	P. vivax (n=102)	P. falciparum (n=96)	Mixed (n=02)	Percentage (%)
Normocytic Normochromic	62	72	02	68
Normocytic Hypochromic	02	04	-	03
Microcytic Hypochromic	28	12	-	20
Macrocytic	04	-	-	02
Dimorphic	06	08	-	07

RBCs were typically microcytic hypochromic (20%), then normocytic normochromic (68%) in that order. In comparison to falciparum, vivax had 14 more cases of microcytic hypochromic blood pictures than did falciparum. Additionally, 2 occurrences of macrocytic blood and 7 cases of dimorphic blood images were seen.

WBC Parameters

Table 15: Total Leucocyte Count

WBC Count /mm3	P. vivax (n=102)	P. falciparum (n=96)	Mixed (n=1)	Percentage (%)
<4000	20	16	-	18
4000-11,000	74	70	-	72
>11,000	08	10	02	10

Majority of the patients had normal WBC count (72%). Reduced counts were seen in 18% of the cases and increased counts in 10%, with near equal distribution in vivax and falciparum malaria.

Table 16: Platelet Count

Platelet count/ mm^3	P. vivax (n=102)	P. falciparum (n=96)	Mixed (n=1)	Percentage (%)
<50,000	30	32	02	32
50,000-1.5 lacs	56	42	-	49
1.5-4.0 lacs	16	20	-	18
>4 lacs	-	02	-	01

Decreased platelet counts were a constant feature of both types of malaria with 81% of cases showing platelets less than 1.5 lacs. Severe platelet reduction (<50,000) was seen in 32 cases.

Table 17: Parasite count with type of malaria

Parasite count in %	P. vivax (n=102)	P. falciparum (n=96)	P. Mixed (n=02)	Percentage (%)
<1	44	12	-	28
1-5	50	68	02	60
>5	08	16	-	12

Parasitaemia of 1-5% was seen in a maximum number of cases (60%). Most of the falciparum infections (42 cases) showed increased parasite count (1-5% or > 5% range). Also 12 cases were with high parasite count (>5%) with 8 of these cases being falciparum malaria.

Discussion

When a female anopheles mosquito transmits malaria, the parasites invade and grow in the circulating red blood cells, resulting in clinical sickness and pathological abnormalities in multiple body organs. Despite major improvements in diagnostic techniques and therapeutic approaches, there are still between 300–500 million cases of malaria worldwide each year, resulting in 1.1–2.7 million deaths. Malaria is endemic in many areas of India. Malaria cases have increased during the past few years. Insecticide-resistant mosquitoes, a rise in chloroquine-resistant malaria, and an increase in falciparum cases relative to vivax cases are some of the factors causing this.

The two most significant haematological changes caused by malaria are anaemia and thrombocytopenia. Malaria exacerbates the population's already precarious health situation in India, where the population's haemoglobin concentration has already decreased due to poor food intake and the load of various illnesses, particularly in youngsters.

The current investigation was conducted between November 2020 and June 2022 and involved 100 smear-positive patients, of which 51 were caused by vivax, 48 by falciparum, and one by mixed infection. In the current study, falciparum (48%), followed by vivax (51%) was the most prevalent

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species of malaria. Jadhav et al. found that vivax was the most prevalent species in their investigations, but Rojansthein et al. and Bashwari et al. found that falciparum prevalence was higher.

The most frequent species found in India is vivax, followed by falciparum. However, the number of falciparum cases has increased recently. This can be ascribed to a number of things, including insecticide-resistant vectors and a failed vector control programme. Any age group might be impacted by malaria. The majority of studies, however, focus more on adults than children. Comparable to Potkar et al., the current investigation included 23 children and 77 adult patients. The current study's age group had a mean of 30.7. The average age ranges in most other research are between 25 and 40. Due to their greater mobility and increased risk of exposure from more outside activity, the adult age group is more affected. The current study included 58% more men than women (42% to be exact). Other studies with similar findings include Bashwari et al. with 75.9% male participants and Erhart et al. with 69% male participants. Less mobility, a society that is dominated by men, and apathy toward treating illnesses in women may all contribute to a higher incidence of male cases.

In cases of malaria, anaemia is frequently discovered, especially in underdeveloped countries. Anaemia's aetiology involves numerous factors. RBCs with parasites are destroyed, non-parasitized RBCs are destroyed more quickly in correlation with illness severity, and bone marrow dyserythropoiesis is present. In the current study, 88% of the cases had anaemia (11 gm %). In previous investigations, Sharma et al. found anaemia in 86.7% of the cases, while Biswas et al. found anaemia in 94.4% of the cases. In investigations done in Saudi Arabia, Bashwari et al. found that 59.2% of cases had anaemia, and Niazi found that 46% did. Depending on where the study was conducted, anaemia caused by malaria infection varies greatly. Only 15% of malaria cases in the study by Richard et al. in London exhibit anaemia. Higher levels of anaemia have been found in studies done in poor nations. The majority of patients in these regions suffer from iron and folate deficiencies as a result of poor food, parasite, and bacterial illnesses, all of which contribute significantly to anaemia. As a result, it is difficult to say how much malaria on its own is responsible for the malaria in these areas. Most research have found anaemia in malaria cases, however the degree of anaemia differs between investigations. Malaria-related anaemia in impoverished nations worsens already low counts, and severe anaemia (5 gm %) is common. In the current study, 11% of individuals had severe malaria, which is a life-threatening consequence. The majority of these significant drops in haemoglobin levels were caused by falciparum malaria.

Leucocytosis was observed in 10% of the cases in the current investigation. Leucocytosis was found to be 7.2% in a study by Bashawari et al. 13.3% and 12.2% of the cases shown by Sharma et al. and Biswas et al. are comparable to those in the current study. Ladhani et al, who only analysed falciparum cases, observed a more pronounced rise, while Echevera et al, who only studied vivax cases, reported a 5% rise. Malaria-related WBC changes are less distinct, and there is a significant amount of heterogeneity among studies. Most patients' total counts typically fall within acceptable ranges. 72% of the participants in the current study had normal counts. Malaria can result in an increase in leucocytosis instances, mainly after subsequent bacterial infections. 7.8% of vivax cases in the current study demonstrate an increase in leucocyte count, while 10.4% of falciparum patients do the same. 18% of the patients in the current study had leucopenia. Leucocytes decrease in 19.6% of vivax patients while leucopenia occurs in 16.6% of falciparum cases. Falciparum malaria was shown to cause 6.6% leucopenia by Sharma et al. and 10.29% by Ladhani et al. Leucopenia was present in 13.3% of all malaria patients, according to Bashwari et al. Leucopenia was found in 29% of the patients of vivax malaria in the study by Echeveria et al. There are variations in the total WBC counts, even though all studies demonstrate some alterations. So, although the degree of change may differ, a change in the WBC count is not unusual for either P. falciparum or P. vivax. The current analysis reveals neutropenia in 13% of cases and increased neutrophils in 6% of instances. Similar results were found in a research by Bashwari et al., which showed neutropenia and neutrophilia to be 8.3% and 11.6%, respectively.

Neutropenia was observed in 14.4% of the cases in the study by Biswas et al., while neutrophilia was present in just 0.6% of the cases. In the current study, lymphocytosis was detected in 8% of cases. Similar to Bashwari et al., Biswas et al. reported 13.6% cases with lymphocytosis while both groups reported 8.5% cases with the condition. In the current investigation, atypical lymphocytes were found in 18% of the cases. Atypical lymphocytes are present in 20.2% of cases in the study by Jain et al. and 38.7% of cases in the study by Bashwari et al. Similar discoveries were made by Roy, who observed high ESR in 81.6% of the falciparum cases. The current investigation reveals raised ESR in 79% of the cases. Due to the acute nature of malaria, patients' ESR readings experience a modest to moderate rise. In the current study, 77% of patients with falciparum malaria and 84% of patients with vivax malaria had thrombocytopenia (1.5 lac individuals). Different investigations have found varying percentages of thrombocytopenia in both vivax and falciparum infections. As in the current study, studies by Bashwari et al. and Jhadav et al. show that thrombocytopenia is more common in vivax malaria, but studies by Horstmann et al. and Erhart et al.69 suggest that thrombocytopenia is more common in falciparum malaria cases. Most investigations have indicated that thrombocytopenia is a frequent finding in instances of both vivax and falciparum malaria. In the current study, 81% of the malaria cases had thrombocytopenia. According to Kueh et al. and Richards et al., thrombocytopenia occurs in 80% of

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cases and 67% of cases, respectively. The most frequent finding is thrombocytopenia, regardless of the type of malaria patients have. The likelihood of malaria increases in patients with acute febrile illnesses in tropical regions, and the presence of thrombocytopenia can serve as a useful clinical signal for initiating treatment. The two kinds of malaria cannot be distinguished by thrombocytopenia, though. Uncertainty surrounds the cause of malarial thrombocytopenia. Theoretical explanations include immune-mediated lysis, sequestration in the spleen, and a dyspoietic process in the bone marrow with reduced platelet synthesis. Malaria has been linked to abnormalities in platelet shape and function, and in some rare cases, the malarial parasites themselves have been observed invading platelets.

Conclusion

Both falciparum and vivax infections can cause a variety of haematological abnormalities, most frequently normocytic normochromic anaemia and thrombocytopenia. Generally speaking, parasite load and the degree of anaemia and thrombocytopenia are correlated. In a patient with a febrile condition, meticulous examination for the malarial parasite is necessary in order to observe thrombocytopenia. White blood cell changes are less pronounced, and the outcomes of different investigations vary. Leucopenia, leucocytosis, and rarely the presence of aberrant lymphocytes are among the reported modifications. Further research and comparison of the white blood cell parameters in immune and semi-immune patients suffering from endemic diseases, as well as other issues that have not yet yielded definitive results, would be helpful. These consist of bone marrow modifications and coagulation characteristics. In conclusion, early malaria diagnosis with consideration for the numerous haematological abnormalities and aggressive, efficient treatment can reduce mortality and avert additional problems.

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