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Original Research Article

Study of Effect of Hematological Parameters in Malaria Infection-Our Experience in a Tertiary Care Centre

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Abstract:

Background: Malaria is a significant health problem in India, being one of the biggest burdens in terms of morbidity and mortality. The mortality rate is high in severe malaria (10-30%), and hematological changes play a crucial role in morbidity and mortality. Monocytes, neutrophils, and lymphocytes play a vital role in the induction of immune responses to malaria infection and their parameters have been proven as predictors of malaria infection and its clinical severity.

Materials and Methodology: This study was done for a period of 2 years i.e., from October 2017 to September2019, in the Department of Pathology, Andhra medical college, Visakhapatnam, during which 107 smear positive cases and 100 controls were studied. In suspected malaria cases Peripheral smear, Thick & Thin Smear & Rapid diagnostic test was done. Rest of the sample was subjected to SYSMEX 5-PART-Hematologyanalyzer to determine the Monocyte to Lymphocyte Count Ratio (MLCR), Monocyte to Neutrophil Count Ratio (MNCR) and Neutrophil to Lymphocyte Count Ratio (NLCR).

Results: Out of 107 smear positive cases, P. vivax is the most common species causing Malaria. 35 cases had severe Malaria with more cases attributed to P. falciparum followed by P. vivax infections. Severe anaemia is seen in 33 cases (30.84%) in which P. falciparum association is predominant. Moderate thrombocytopenia is the most common presentation. Severity of anaemia and thrombocytopenia correlated with parasitemia. Among leucocyte count ratios – there is significant correlation between Monocyte to Lymphocyte Count Ratio (MLCR) and parasitemia.

Conclusion: The grade of anaemia and thrombocytopenia increases with the severity of malaria especially in P. falciparum infection. MLCR (Monocyte Lymphocyte Count Ratio) can be used as a screening tool/biomarker along with clinical and other haematological parameters to predict the severity of malaria.

Keywords: Severe Malaria, Leucocyte count Ratios, Parasitaemia.

Introduction

Malaria is a significant health problem in India, being one of the biggest burdens in terms of morbidity and mortality [1]. Clinical manifestations of malarial illness are due to the asexual forms of the malaria parasite, which infects red blood cells and makes malaria a viable multisystem disease, as every organ of the body is reached by the blood [2,3].

According to the World Health Organization, World Malaria report 2018, India accounts for 4% of the global malaria burden and contributes 87% of the total malaria cases in Southeast Asia [4]. The mortality rate is high in severe malaria (10-30%), and these hematological changes play a crucial role in morbidity and mortality. During malaria infection, parasites and red blood cells come under oxidative stress, and there is host immune response in terms of changes in Monocyte and Lymphocyte population in an attempt to protect the red blood cells [5,6].

Many studies have investigated the role of peripheral blood leukocyte ratios: Monocyte to Lympho-cyte Count Ratio (MLCR), Monocyte to Neutrophil Count Ratio (MNCR) and Neutrophil to Lymphocyte Count Ratio (NLCR) in different clinical entities like critical illness, sepsis, tuberculosis, myocardial infarction and also in malaria. These monocytes, neutrophils, and lymphocytes play a vital role in the induction of immune responses to malaria infection, and their

parameters have been proven as predictors of malaria infection and its clinical severity [7-9].

Materials & Methods: This study is an observational study for a period of 2years with a sample size of 107 cases and 100 normal controls

Inclusion Criteria: Newly diagnosed cases of malaria.

Exclusion Criteria: History of antimalarial therapy

Methodology: Clinical data recorded as per proforma. 2ml Blood was collected in EDTA vacutainer. In Sus-pected Malaria cases Peripheral smear, Thick & Thin Smear & Rapid diagnostic test was done. Peripheral smear was stained with Leishman. Thick and thin smear stained with Giemsa & Jaswanth Singh Bhattacharya stains.

Rest of the sample was subjected to SYSMEX 5-PART-HEMATOLOGYANALYZER to determine the ratios given below. The Monocyte to lymphocyte count ratio (MLCR) and Monocyte to neutrophil count ratio (MNCR) and Neutrophil to lymphocyte count ratio (NLCR) were calculated and compared with normal controls. Statistical analysis is done using Microsoft Excel 2010 and Statistical Package for the Social Sciences (SPSS) for Windows version 21.0 (SPSS Inc., Chicago, IL).

Calculation of Parasitemia in Thick & Thin Smears:

A. Thick Smear: With the thick smear, a minimum of 100 fields are examined for the presence of blood para-sites under oil immersion objectives. The parasites are counted against 500 leukocytes and parasites per micro-litre of blood calculated against the total white cell count obtained from the full blood count using the formula:

Parasites / μL = No. of parasites X patient's WBC count 500

B. Thin Smears: Record the total number of parasitized red cells and the total number of red cells counted in the 20 fields of thin film. Calculate the parasite density from the formula:

Parasites / μL = No. of parasitized red cells X patient's RBC, No. of red cells counted

(OR)

Calculate number of infected RBCs in 1000 RBCs and calculate its percentage, No. of infected RBC in 1000 RBCs /10 = ---- %

Results: A total of 107 patients and 100 controls were included in the study. P. vivax (54.21%) is the most common species with slight male preponderance (52.4%).

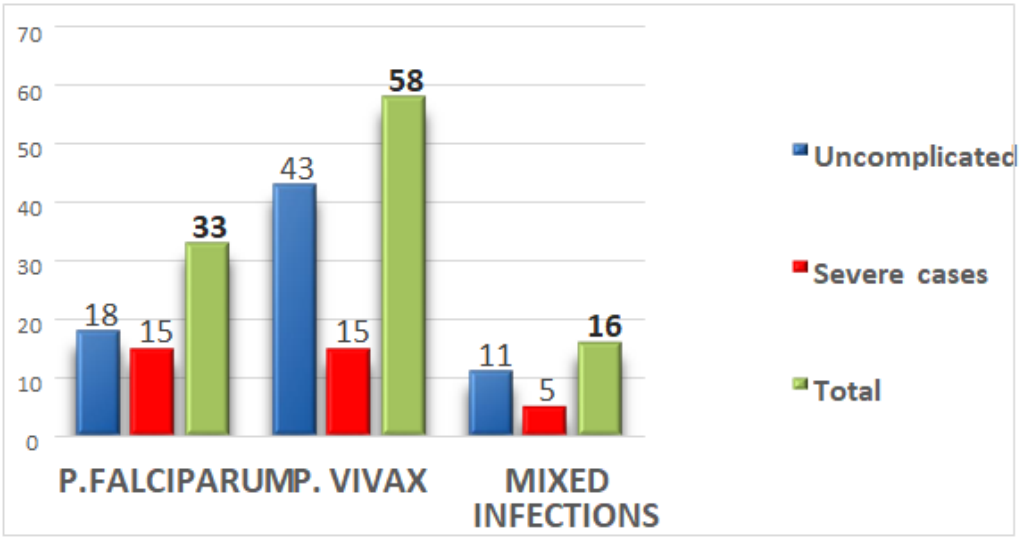
Table 1: Species wise distribution of Anemia (n=107)

Anemia	P.F	P.V	P.F + P.V	Total	%
Normocytic (>11)	7	26	2	35	32.71
Mild (10-10.9)	4	7	0	11	10.2
Moderate (7.0- 9.9)	9	14	8	31	28.9
Severe (<7)	13	11	6	30	28.2

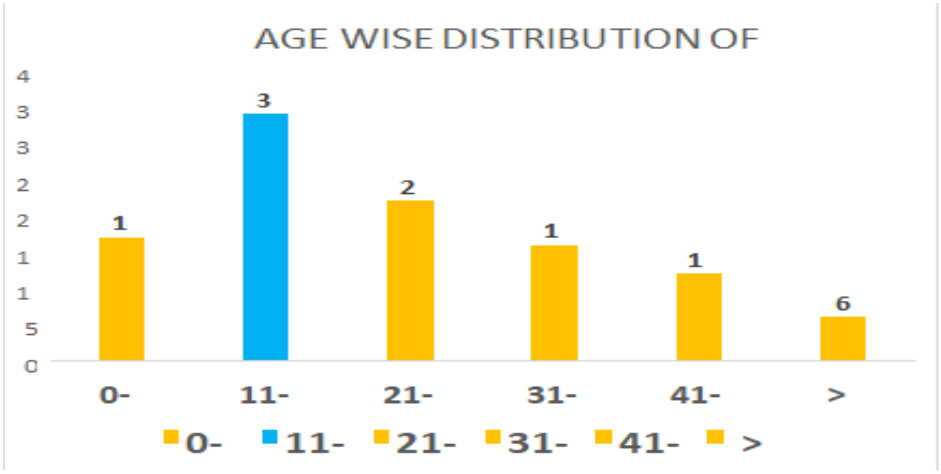
Based on WHO guide lines of criteria for severe Malaria (>10 percentage of parasitemia , Hematocrit < 15 , Hemoglobin <7g/dl) ; 35 cases(35/107) of severe malaria were diagnosed out of which 15 cases of P. falciparum, 15 cases of P. Vivax and 5 cases of Mixed infections(Bar diagram1).

Patients of all age groups were seen; youngest was 4 months old female child with Mixed infection and oldest was 75 year old male with P. falciparum infection (bar diagram 2).

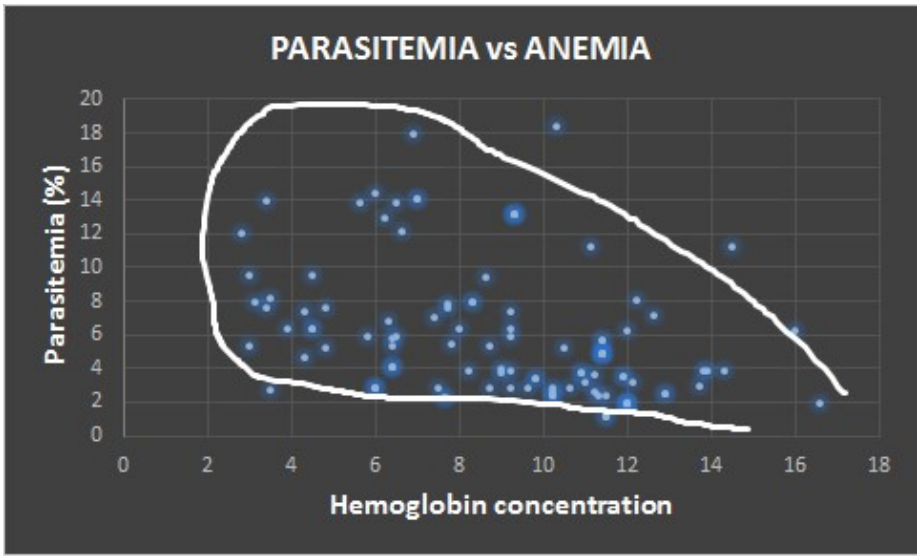
11-20 years age group is the most common age group infected in the present study. Severe anemia is seen in 33 cases (30.84%) in which P. falciparum species accounts for 13 cases, P. vivax 11 cases and mixed infections 6 cases. Degree of parasitemia correlates with degree of Anemia (Scattergram 1). Various leucocyte count ratios MLCR, NLCR and MNCR were increased when compared to normal controls. NLCR & MLCR is significantly raised in P. falciparum and MNCR is significantly raised in Mixed infections. There is significant correlation between MLCR & MNCR among cases and controls. ($p<0.05$) t-test.* but no significant correlation between NLCR among cases and controls. ($p>0.05$) t-test.*(tab 2).



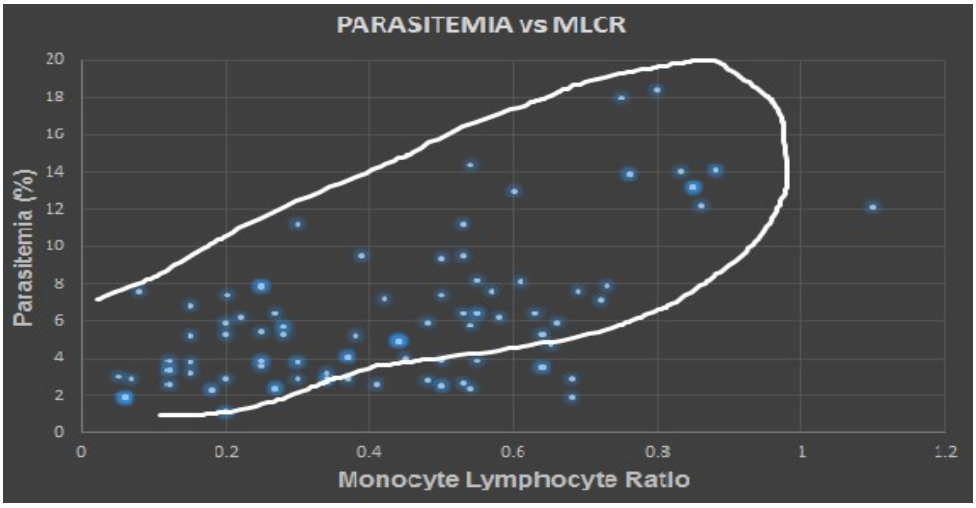
Bar diagram 1: Plasmodium species & Parasitemia association with Severe Malaria (n=107)



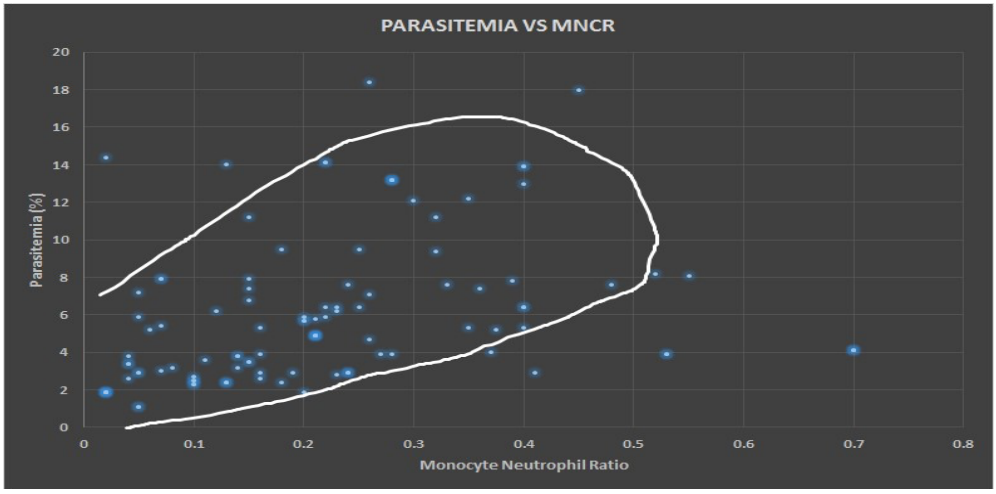
Bar diagram 2: Age wise distribution of malaria (n=107)



Scatter diagram 1: showing relation of Paraistemia vs Anemia (n=107)



Scatter diagram 2: relation of Parasitemia with MLCR



Scatter diagram 3: relation of Parasitemia with MNCR

Decrease in platelet count is seen in 85.04 % cases. Moderate thrombocytopenia is the most common presentation seen in 36 (33.64%) cases followed by severe thrombocytopenia seen in 30(28.03 %) cases and mild thrombocytopenia in 25 cases. (tab5) Severe thrombocytopenia cases most associated with P. falciparum infection.

Platelet Parameters like MPV, PDW and P-LCR were increased, and this increase is more predominant with P. falciparum. PCT is decreased in all species of Malaria. Degree of parasitemia correlated with extent of thrombocytopenia.

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Table 2: Sensitivity and Specificity of Leucocyte count Ratios

	Cut Off Value	Sensitivity	Specificity	p- value
NLCR	≥ 2.01	57%	70%	p>0.05
MLCR	≥ 0.19	83%	71%	P<0.05
MNCR	≥ 0.065	84.1%	76%	P<0.05

Table 3: Association of thrombocytopenia with Malaria (n=107)

Alterations In Platelet Count	Species			Total	%
	P. f	P. v	Mixed		
Normal (> 1.5 lakhs)	5	11	0	16	14.95
Mild thrombocytopenia (1-1.5 lakhs)	2	19	4	25	23.36
Moderate thrombocytopenia (0.50-0.99lakhs)	10	20	6	36	33.64
Severe thrombocytopenia (<0.50 lakhs)	16	8	6	30	28.03

Photo Micrographs

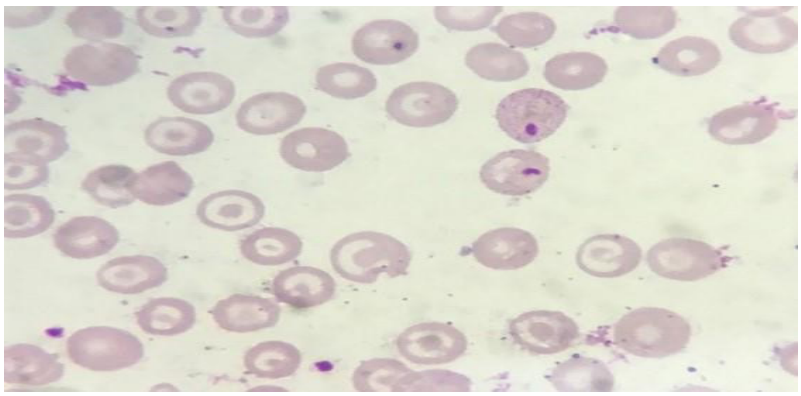

Figure 1: Peripheral smear showing P.Vivax ring forms

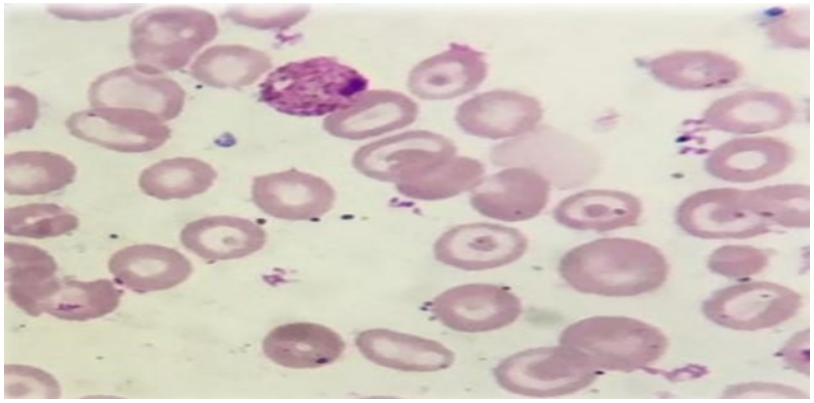

Figure 2: Peripheral smear showing trophozoite of P. Vivax

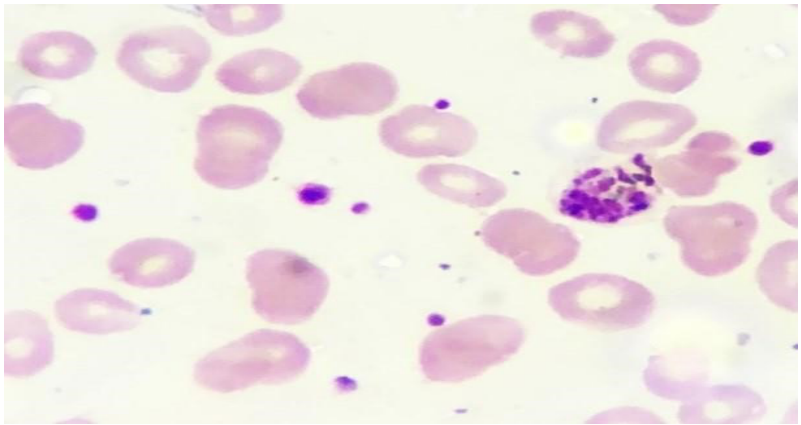

Figure 3: Peripheral smear showing P. vivax schizont forms

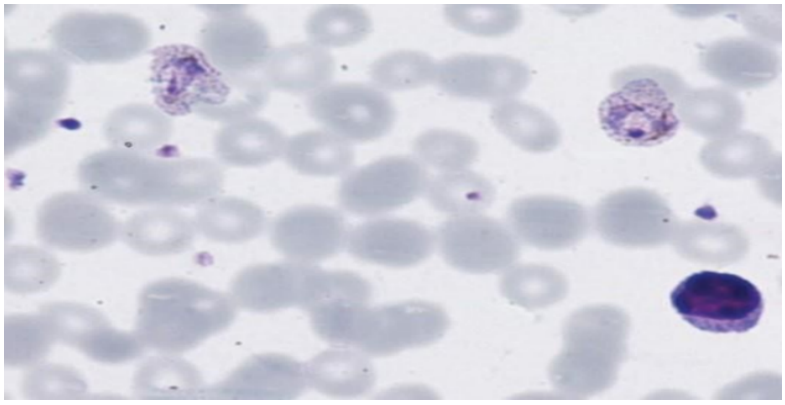

Figure 4: Peripheral smear showing P.vivax Late trophozoite forms

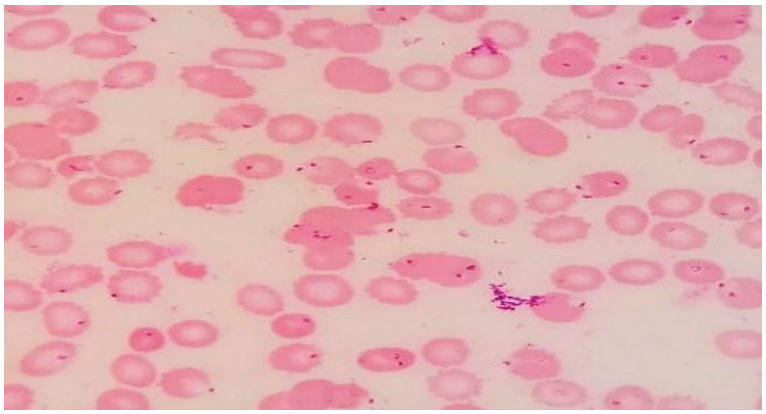

Figure 5: Peripheral smear showing ring forms of P. falciparum

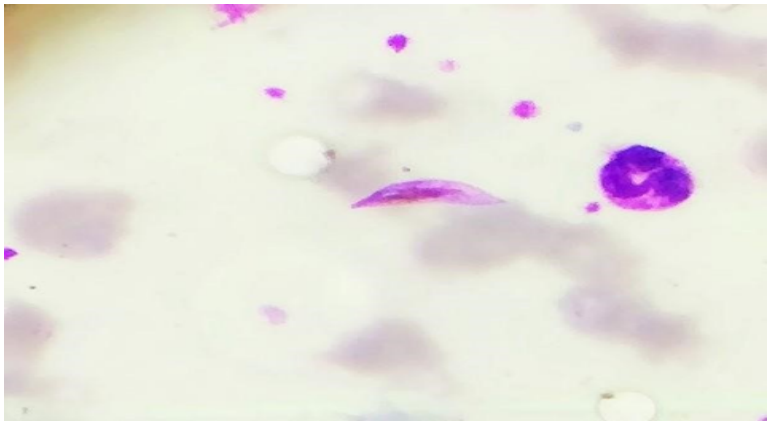

Figure 6: Peripheral smear showing P.falciparum gametocyte

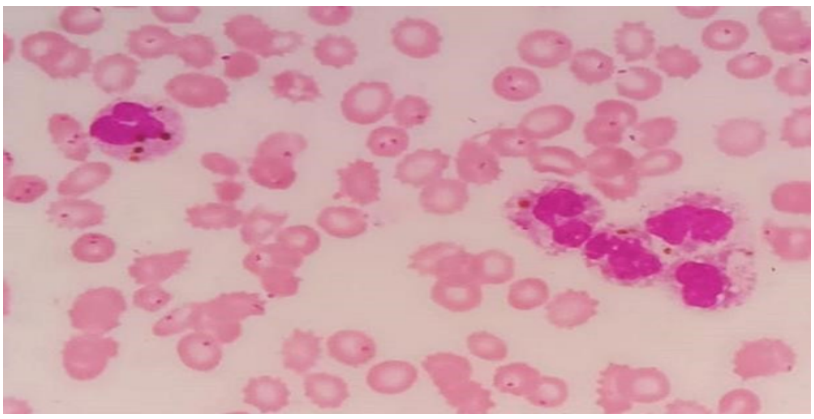

Figure 7: Peripheral smear showing Monocytes containing hemozoin pigment in P.falciparum infection

Discussion

Malaria is a life threatening, infectious disease caused by Plasmodium species which is transmitted by female anopheles mosquito. It is a blood parasite involving circulating RBCs and causes hematological and systemic changes which can lead to lethal complications.

Despite new advances and interventions, the incidence of malaria is 300-500 million cases and 1.1 -2.7 million deaths annually [1]. Anemia and thrombocytopenia are the most important hematological alterations encountered in malaria. Early diagnosis and intervention helps in reducing disease burden and mortality.

The present study was done for a period of 2years i.e., from October 2017 to September 2019, in a Tertiary care center ,during which 107 smear positive cases and 100 controls were studied.

The most common species of malaria in the present study is P. Vivax followed by P. falciparum. In the studies conducted by Erhart et al [10], Jadhav et al [11], Surve KM S et al [12], S. Sumathi [13], Motchan et al [14], Philipose CS et al [15] and Deepti Arora et al [16] P. vivax was the most common species while the studies done by Rojansthein et al [17], Bashwari et al [18] and Khuraiya P et al [19] reported higher P. falciparum prevalence.

In India P. vivax is the most common species encountered followed by P. falciparum. However in recent years there has been an upswing in the P. falciparum and mixed infections. Various factors can be attributed to this are vector resistance to insecticides, drug resistance to chloriquine and failure of vector control program in rural areas.

Sex distribution: Among 107 cases, 56 were males (52.74%) and 51 were females (47.25%). M: F ratio is 1.09:1. Males are more commonly effected than females which is correlating with the studies done by Erhart et al [10], Bashwari et al [18] and Deepti Arora et al 16(1.34:1) and S. Sumathi et al [13], and Motchan et al [14].

Plasmodium species association with severity: Among 107 cases, 35 cases met the criteria for Severe Malaria in which P. falciparum accounts for 15 cases (45.4%), P.vivax for 15 (25.8%) cases and 5(31.2%) cases of Mixed infection.

P. falciparum is the most common species causing severe malaria in the present study which is correlating with the studies done by Bashwari et al [18] and Agravat et al [20]. Parasites causing severe malaria have a greater multiplication potential than those causing uncomplicated infections. [21] Adherence of infected red blood cells to the endothelium of small blood vessels compromising blood flow through tissues, and the production of proinflammatory cytokines [22]. Cyto-adherence of parasitized red cells may be influenced by the virulence of different strains of parasite [23]. Lack of this protective immunity and differences in HLA antigens may play a role in host predisposition to severe disease.

Comparison of Age distribution: Mean age group involved in the present study is 11-20 years which is correlating with the studies done by Deepti Arora et al [16], Khuraiya P et al [19].

Out of 35 cases of severe malaria, 11-20 years is the most common age group infected by P .falciparum & P. vivax with equal distribution. Mixed infections are seen more commonly in the age group of 1-10 and 21-30 years with equal distribution.

The higher incidence of plasmodium prevalence in the young adults is due to premunition which is attained in late adulthood and increased outdoor activities in younger age group 19.

Anemia & Malaria: Anemia is the most common presentation in this study with 72 (67.2%) cases which is correlating with the studies done by Khuraiya P et al [19] and Baswari et al [18]. In the studies done by Deepti arora et al [16], Biswas et al [24] and Sharma et al [25] showed a high prevalence of anemia.

Severe anemia is seen in 33(30.84%) cases which is correlating with the study done by Khuraiya P et al 19 which showed severe anemia in 34.6% cases. Studies of Baswari et al [18] and Sharma et al [25] showed fewer cases with severe anemia i.e., 5.5% and 8 % respectively. Anemia in malaria is multifactorial in origin. Geographical location, nutritional status, hemolysis of parasitized RBCs as well as normal RBCs, genetic factors, oxidative stress and bone marrow dyserythropoiesis play a major role in anemic status of the patient [26 ,27].

Parasitemia and Anemia: Parasitemia and degree of anemia correlated positively ,i.e., with increase in parasitemia there is increase in severity of anemia ($p<0.05$).This is in correlation with the studies done by Kotepui et al 6 and Yeka A et al [28] .

This increase in severe anemia associated with high parasitemia (>10%) is due to increased hemolysis of infected RBCs, splenic and reticular hyperactivity and other genetic factors [29].

Comparison of leucocyte count ratios with normal controls: When compared with normal controls there is Increase in Leucocyte count ratios i.e., MLCR, MNCR & NLCR in the present study. MLCR & NLCR ratios were more markedly increased in P. falciparum infection followed by P. vivax infection while MNCR is increased in mixed infections followed by P. falciparum. Severe

malaria showed higher MLCR, NLCR and MNCR values when compared to Uncomplicated/less severe malaria. This increase in MLCR is well correlating with the studies done by Antwi-Baffour et al [29] and Warimwe et al [30].

This increase in Leucocyte count ratios is due to the course of malaria infection in which the parasites and erythrocytes come under oxidative stress and in an attempt to protect erythrocytes there is host immune response in terms of changes in monocyte and lymphocyte population. This variation in monocytes and lymphocytes in peripheral blood reflect the state of an individual's immune response to infections 5 .This high monocyte to lymphocyte ratio might indicate a predominantly pro-inflammatory immune response that renders an individual susceptible to clinical malaria and increased parasitemia [29].

NLCR values in the present study correlating with the study done by Wolfswinkel et al [7] and Louis dias et al [31]. NLCR is attributed to the increase in immature neutrophil fraction due to premature release from bone marrow and circulating pool decreases due to shift of neutrophils to margination pool [32]. As Monocytes and Neutrophils are the principle cells involved in malaria pathogenesis and clearance of parasitemia the MNCR ratio is increased in severe malaria [31].

Parasitemia vs Leucocyte count Ratios: MLCR and MNCR correlates positively with Parasitemia i.e., with increase in MLCR and MNCR there is increase in Parasitemia which is correlating with the studies done by Antwi-Baffour et al [29] and Louis das et al [31] respectively.

In the present study, MLCR, NLCR and MNCR has a sensitivity of 83%, 57%, and 84.1% respectively and Specificity of 71%,70% and 76% respectively. MLCR and MNCR in the present study correlating with the studies done by Louis das et al [31] and Wolfswinkel et al [7] respectively with $P<0.05$.

Which is showing significant correlation while NLCR ratio correlates with Kotepui et al [6] with p >.05 which is not significant. Leucocyte count ratios of MLCR and MNCR has high sensitivity and high specificity and NLCR has low sensitivity and high specificity

Thrombocytopenia and malaria: In the present study the percentage of patients showing thrombocytopenia (<1.5 lakhs/mcl) were 84.4% in case of falciparum malaria and 81.03% in case of P.vivax. Thrombocytopenia is more in cases of falciparum malaria in the present study which correlates with the studies conducted by Horstmann et al [33] and Erhart et al [10].

Among 35 cases of severe malaria, 12 cases (80%) of P. falciparum show severe thrombocytopenia .The mechanism of thrombocytopenia is uncertain. Immune mediated lysis, sequestration in the spleen and a dyspoietic process in the marrow with diminished platelet production have all been postulated. Platelet indices like Mean Platelet Volume (MPV), PDW (Platelet distribution width), P-LCR (Platelet Large cell Ratio) increased in malaria and these changes are more significant in P. falciparum infection while PCT(Plateletcrit) is decreased in malaria infection. Parasitemia vs Thrombocytopenia:

In the present study high parasitemia is associated with marked thrombocytopenia. Degree of parasitemia correlated with severity of thrombocytopenia. Ladhani et al [35] study show thrombocytopenia strongly associated with degree of parasitemia. In studies carried out by Rojansthein et al [7] thrombocytopenia was observed in both Plasmodium Vivax and Plasmodium falciparum malaria and correlated with the degree of parasitemia. In studies carried out by Horstmann et al [33] correlation was found between platelet counts and high counts of malaria plasmodia in P. falciparum and P. vivax.

Conclusions & Summary

- P. Vivax is the most common species in the present study.
- 11-20 years of age group is more prone to malaria infection.
- The grade of anemia and thrombocytopenia increases with the severity of malaria especially in P. falciparum infection.
- In cases of severe malaria both species are to be considered as there is alarming rise in P.vivax associated severe malaria.
- Monocyte to lymphocyte Count Ratio (MLCR) directly correlated with parasitemia.
- MLCR (Monocyte Lymphocyte Count Ratio) can be used as a screening tool/biomarker along with clinical and other hematological parameters to predict the severity of malaria.

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