Study on prevalence and hematological parameters in Malaria infected patients visited local hospitals of Rawalpindi, Pakistan

By

Nayyar Sarfaraz

Department of Zoology

Faculty of Biological Sciences

Quaid-i-Azam University

Islamabad

2023

Study on prevalence and hematological parameters in Malaria infected patients visited local hospitals of Rawalpindi, Pakistan

A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Philosophy

In

Parasitology

By

Nayyar Sarfaraz

DEPARTMENT OF ZOOLOGY
QUAID-I-AZAM UNIVERSITY
ISLAMABAD
2023


بسم الله الرحمن الرحيم

CERTIFICATE

This dissertation "**Study on prevalence and hematological parameters in Malaria infected patients visited local hospitals of Rawalpindi, Pakistan**" submitted by **Nayyar Sarfaraz**, is accepted in its present form by the Department of Zoology, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan as satisfying the thesis requirement for the degree of Master of Philosophy in Parasitology.

Supervisor

Dr. Kiran Afshan
Associate Professor

External Examiner

Chairperson

Dr. Amina Zuberi
Department of Zoology

DECLARATION

I hereby declare that the material and information presented in this thesis in my original work. I have not previously presented any part of this work "**Study on prevalence and hematological parameters in Malaria infected patients visited local hospitals of Rawalpindi, Pakistan.**"

Nayyar Sarfaraz

DEDICATION

Dedicated to my beloved parents, my brothers and dear husband.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEDGMENT

Praise is to **Almighty Allah**, the cherisher and sustainer of the worlds, the most merciful, the most beneficent, who showed his countless blessing upon us. Almighty Allah enabled me to compile my humble endeavor in the shape of this dissertation. All respect goes to the Holy prophet **Hazrat Muhammad (S.A..W)** whose teaching strengthen our faith in Allah and guiding the mankind, the true path of life, who is the source of knowledge a wisdom of entire humanity, who clarified that pursuit of knowledge is a divine commandment.

I would like to express my gratitude to all those who gave me the possibility to complete this thesis. I have a great exhilaration for my supervisor **Dr. Kiran Afshan** for her valuable guidance, constructive criticism and encouragement throughout the course of my research work and I am indebted to chairperson **Dr Amina Zuberi** for her encouraging behavior and obliged to her for extending the research facilities of the department to accomplish this work.

I also thank my dear mother **SHUGFTA NISAR** and father S**ARFRAZ AHMED** for their continuous support and their pray help me to reach this destination.

I owe a deep sense of gratitude to my husband **ATIF HANIF** for guiding me during my research work. His great support and help enabled me to complete my research.

My deepest appreciation and thanks to my elder brother **KAMRAN SARFRAZ(LATE)** for their motivation. His motivating words helped me in completing my M.Phil.

I am also thankful to my other brothers **USMAN SARFRAZ** and **ZEESHAN SARFRAZ** for their help **a**nd support and special thanks to **NOUMAN SARFRAZ for** helping me in compiling my whole research writing.

Also thanks to **ANUM BJI** and R**EHANA BJI for** helping me in data collection.

ABSTRACT

Background: Malaria is a serious health problem in poor countries, and a high number of mortalities are associated with various hematological complications if untreated. Malaria is prevalent in Pakistan with various hematological complications. The present study aimed to determine the malarial prevalence and hematological parameters in infected patients in Rawalpindi districts.

Methods: A cross-sectional study comprised of 500 participants was conducted from April 2021 to September 2022. The study includes participants belonged to all age groups from six public healthcare facilities in Rawalpindi. Participants with hemolytic diseases or any other hematological disorders were excluded from the study. Microscopic Giemsa-stained slides were prepared. Blood was also tested for anemia, leukopenia, leukocytosis, and thrombocytopenia. Chi-square analysis and t-test was performed in SPSS ver.20.

Results: Out of 500 participants, 181(36.2%) were infected with malarial infection. Middle-aged people 21-40 years old had significantly ($P \leq 0.05$) higher prevalence (16.6%) to malarial infection. The infection was significantly ($P \leq 0.05$) more prevalent (53.1%) in females compared to male participants. The RBCs level was not differed significantly ($P \geq 0.05$) between malarial infected males and females as compared to non-infected. However, a significant ($P=0.001$) decrease in platelets count was recorded. Other hematological parameters i.e. hemoglobin, Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC) significantly ($P \leq 0.05$) increased in infected patients, while lymphocytes, monocyte and eosinophil decrease significantly ($P \leq 0.05$).

Conclusion: The finding suggested high malaria spread in Rawalpindi district. Further, studies on malarial control and implementation of intervention strategies are required.

INTRODUCTION

The most serious and widespread protozoan illness of humans is malaria. It is primarily found in tropical and subtropical regions of Africa and Asia. Malaria has been shown to have a major global impact on morbidity and mortality. One of the most common human infections, malaria is found in 91 countries, predominantly in developing ones. Of the world's population more than 40, lives in malaria-endemic regions, where 300 to 500 million infections and 1.5 to 2.7 million fatalities are expected annually (Shamim Akhtar *et al*., 2012). When there is severe infection (parasitemia > 5%), the morbidity rate is typically higher (20%). In the liminal space between parasitology and haematology, malaria has long played an important role. Malaria was described as a "typical blood illness" in the 1930s by a classic European haematology textbook as having symptoms like fever, splenomegaly and anaemia. It is currently thought to be a typical example of hemolytic anemia in more recent hematology. As parasites of the blood for most of their complex life cycle, they expectedly induce hematological changes, which play a significant role in serious complications (Tuteja and Kotepui, 2007).

1.1 Life Cycle

Malaria parasite needs two types of hosts, an invertebrate also called definitive and a vertebrate also called intermediate host. The mosquito which is infected injects secretion (saliva) containing small 10 to 15 elongated sporozoites into the blood stream of vertebrate whereas taking blood. The sporozoites migrate towards liver in 5-7 days and develop into schizont inside liver the cells (*P. falciparum*). Inside schizont about 30,000 merozoites are present. On schizont rupturing these merozoites discharged into the blood. Every sporozoan can infect a erythrocyte. Within erythrocyte sporozoan develops and divides, to make 8 to 32 parasites. When erythrocyte ruptures, they become free and attack on another erythrocyte (Pinder *et al*., 1973). These rounds repeat within erythrocyte. In this way parasites increase their number within the infected human and resulting in clinical health problem. Some parasites within the red cells don't divide and

undergo sexual stages male and female gametocytes which are swallowed in *Anopheles* mosquitoes and inside mosquito body further progress takes place (Schmidt and Roberts, 1996). The male gametocytes which are also called microgametocytes are highly active, separate themselves, and go away and link to protozoa. The female gametocyte also called macro-gametocytes go through nuclear division and converted into gametes. Male and female gametes fuse together and fertilization takes occurs. The zygote after fertilization passes through different developmental series such as ookinetes and oocyte. The zygote nucleus repeatedly divides and thousands of sporozoites that are minute thread like structures are formed. In the insect's haemocoel the sporozoites become free and through circulation they reach to all body parts. Some of them enter the salivary glands cells then pass through and reach the lumen of salivary ducts. The infected mosquitos when bites a healthy person it transfer the sporozoites with saliva and the new asexual cycle starts (Blaklock and South well, 1987).

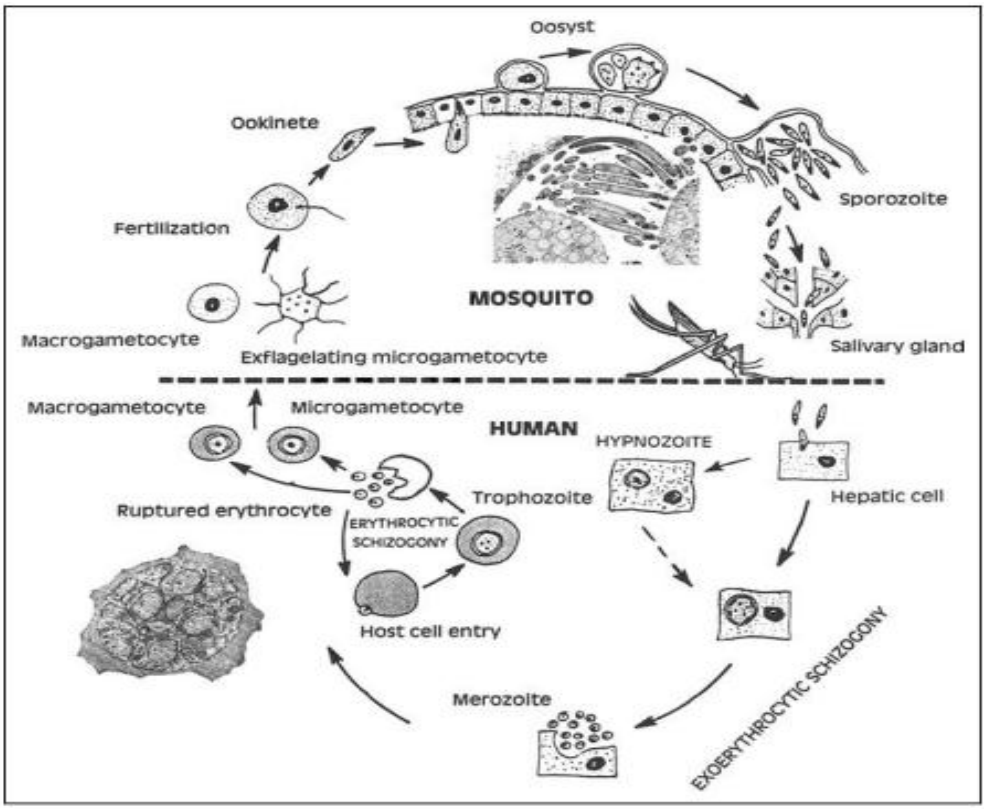

Figure 1.1: Life cycle of *Plasmodium* and human pathogenicity.

1.2 Pathogenesis

Some of the hematological abnormalities that have been documented to typically accompany malaria infection include anemia, splenomegaly, thrombocytopenia, mild-to-moderate atypical lymphocytosis, and occasionally disseminated intravascular coagulation (DIC). Leucopenia and leukocytosis reports have also been made. Moreover, leucopenia and leucocytosis have been reported. There have also been reports of neutropenia, eosinophilia, neutrophilia, and monocytosis as other hematological reactions to malaria. Many researches on hematological anomalies have been carried out in areas where the disease is endemic, some only on children and others just on people with severe malaria. There aren't many research that have been done on patients returning from their endemic nations or on non-immune or semi-immune travelers returning from endemic locations (Bashawr *et al.,* 2002). Anemia is a natural byproduct of infection and hematologic abnormalities are characteristics of infection (Kimbi *et al.,* 2013). An established characteristic of malarial infections is changes in blood cell counts. Major cell lines affected by these modifications include leukocytes, thrombocytes, and red blood cells (RBC). In the course of a malaria infection, hematological abnormalities such anemia, thrombocytopenia, and leukocytosis or leucopoenia are well known. The degree of malarial endemicity, background hemoglobinopathy, dietary state, demographic variables, and malaria immunity all influence these changes (Erhart *et al.,* 2004).

1.3 Global Prevalence

Malaria is currently one of the most serious infections impacting mankind. Moreover, 300 to 500 million cases of malaria are thought to occur each year, resulting in 1.5 to 2.7 million fatalities, and over 40% of the world's population resides in these areas. The death rate in cases of severe malaria is often high (20%) (parasitemia > 5%) (Abro *et al.,* 2008). According to the most current Global Malaria Report, there were 247 million cases of malaria in 2021 compared to 245 million in 2020. Malaria deaths were projected to drop from 625,000 in 2020 to 619,000 in 2021. The COVID-related setbacks led to an additional 13 million cases of malaria and 63 000 fatalities from malaria throughout the two pandemic peak years (2020–2021). In the WHO African Area, malaria still has a disproportionately high global burden. In the Region in 2021, 96% of all malaria cases

and 95% of all malaria deaths occurred. Children under the age of five were responsible for slightly more than 80% of all malaria-related deaths in the area. In four African countries, there were just over half of all malaria cases.

1.4 Prevalence in Pakistan

Pakistan has a tropical climate and a sophisticated irrigation system, which causes enormous ditches to fill with stagnant water after monsoon rains, which is ideal for mosquito breeding. Although year-round transmission, malaria's severity increases after rain (Zeb *et al.,* 2015). Sixty percent of Pakistan's population, or 161 million out of 95 million people, reside in areas where malaria is endemic. Following eradication attempts in the 1960s, malaria reemerged as an epidemic in the 1970s. Recent floods, which affected roughly 20 million people in over 60 regions, can be partially blamed for an increase in malaria cases. According to the malaria control program, Pakistan has 500,000 malaria infections and 50,000 fatalities annually, with 37% of cases anticipated to occur in areas near the borders with Iran and Afghanistan (Mukhtar *et al.,* 2006). Malaria transmission is assumed to be uneven since *P. vivax* transmission peaks in June and September and in April and June, respectively. In Pakistan, *P. falciparum* transmission occurs during the months of August and December (Bouma *et al.,* 1996; Khattak *et al.,* 2013).

1.5 Current Investigation

Hyperparasitemia has been one of the World Health Organization's severe *P. falciparum* malaria criteria for more than 20 years (WHO 1990). Previous research has demonstrated a relationship between parasite density and the severity of malarial illnesses (Tangpukdee *et al.,* 2012). Death rates are also correlated with the degree of parasitemia; for example, those patients who have the highest parasite densities also have the highest mortality rates (Murthy *et al.,* 2000). Similar to how anemia is seriously risked by excessive parasitemia brought on by Plasmodium infection. Due to the fact that a malaria infection causes excessive RBC hemolysis, which might cause anemia (Ekvall, H, 2003). In high parasitemia stages, thrombocytopenia and low platelet levels were also observed in the majority of malaria patients (Manas Kotepui *et al*., 2015).

Thrombocytopenia and anemia are prevalent in patients with *Plasmodium falciparum* malaria. Nonetheless, the majority of investigations on hematologic abnormalities have been carried out in endemic nations where frequent malaria attacks and high prevalences of nutritional inadequacies lead to hemoglobinopathies. There haven't been many investigations on non-immune and semi-immune hematologic disorders. Men's hemoglobin levels during anemia are 12 g/dL, while women's levels are 10 g/dL. Anemia and malaria were only linked in *P. falciparum* infections, and there was no connection between the severity of anemia and the level of parasitemia. Low level and statistically significant hematocrits were discovered in *P. vivax*-infected patients. Thrombocytopenia was observed in patients with *P. vivax* and *P. falciparum* malaria, and it was correlated (r = 0.974) with the degree of parasitemia. In *P. vivax* malarial patient's plasmin activity was normal but it was significantly high in *P. falciparum* patients having parasitemia more than 5 percent (Rojanasthien *et al*., 1992).

An earlier study found that anemia is the most common haematological abnormality. Although spontaneous bleeding is rarely a side effect of malaria, it frequently occurs in thrombocytopenia. Anemia was present in 69% of the patients, and among them, 14% had severe anemia (hb > 6 gm%). Sharma *et al* findings have revealed a higher overall incidence of anemia, at 86.7%. Only falciparum malaria patients were included in their analysis, which helps to explain why the incidence has increased. They only consider the falciparum cases study, which showed an incidence of 80%. Just 49 of the 69 anemic patients displayed splenomegaly, suggesting that anaemia may have reasons other than splenic sequestration (Kashinkunti and Alevoor, 2014).

A week following the initial presentation, the malaria-related drop in hematocrit was at its largest, with a mean fall of 10% in RBC mass and a drop in hematocrit exceeding 25% in nearly 7% of patients, according to prior studies. Around 25% of the hematocrit drop caused by malaria occurred before presentation, and the remaining 75% occurred after therapy. The youngest age groups, those with high parasite burdens, and those who had a fever that persisted for longer than two days before to presentation all experienced the most severe haematological injury. A delay in the treatment response enhanced the risk of malaria-related complications, in line with past findings (Bloland PB

et al., 1993). Malaria results in dyserythropoiesis, and reticulocytosis is repressed during the acute stage of the illness. The primary cause of anemia in malaria is hemolysis, therefore this does not, however, explain how rapidly it manifests. This is often attributed to the loss of parasitized erythrocytes, although there is also evidence that unparasitized erythrocytes degrade more quickly (Ric N, 2001).

The climate of Pakistan's ranges from tropical to temperate with dry conditions along the southern coast, and elevation ranges from sea level to nearly 9,000 meters. In Pakistan *Plasmodium vivax* causes about 64% of infections and *Plasmodium falciparum* causing 36% of infections. These two are prevalent *Plasmodium* species in Pakistan. Primarily malaria is found in Sindh, Khyber Pakhtunkhwa, Balochistan, and the Federally Administered Tribal Areas. Malaria transmission is thought to be unbalanced (Bouma *et al*., 1996; Khattak *et al*., 2013). Therefore, the present study was aimed to investigate the prevalence of malaria in district Rawalpindi along with hematological profile. The objectives of present study were:

1. To determine the prevalence of malaria among patients visited local hospitals in Rawalpindi.
2. To find the association of malarial infection with hematological parameters.

MATERIALS AND METHODS

2.1 Study Areas

Data collection and a cross-sectional epidemiology study were carried out in the district of Rawalpindi from April to September 2022. Six public healthcare facilities in Rawalpindi provided the information. They included the M care Lab, Maryam Hospital, Mega Hospital, Cantonment Hospital, Nusrat Hospital, and Aman Hospital. Malaria caused by *Plasmodium vivax* is continuously transmitted in Rawalpindi.

Fig. 2.1: Map of Pakistan showing Rawalpindi district study area.

2.2 Clinical recruit

Patients who gave their consent to participate in the study had *P. vivax* malaria diagnoses and were treated at medical facilities only for symptoms of malaria. The patients' ages ranged from one to 82. Those older than 15 years old were categorized as adults. The doctors of the appropriate hospitals did confirm all malaria symptoms in the recruited patients for the study controls. Government-accredited healthcare facilities with

comparable tools and methodologies were used in all of the hospitals and Polyclinics in this study.

2.3 Blood film and Slide examination

Hematological parameters for the patient were established. For each patient, the Complete Blood Counts were calculated, and malarial parasite microscopy was also carried out. Three-part differential counter CBC was performed (KX-21). In each case, a white blood cell differential was also performed. Leishman's stain was used to create and color the periphery streaks. For the purpose of identifying malarial parasites, the slides were examined. All smears that tested positive for malaria were examined to confirm the parasite's identity and to determine the platelet count. The gold standard for diagnosing malaria was a smear test for the presence of malarial parasites.

2.4 Data analysis

The Chi-square test was used for the statistical analysis. Hematological parameters were subjected to an independent t-test, and the data's mean and standard error values were provided. Using SPSS 20, descriptive and comparative analysis was performed (IBM). The cutoff for statistical significance was p 0.05.

RESULTS

A total of 500 patients were recruited for this study. The 181 patients out of 500 (36.2%) were verified malaria cases. Of these 319 patients (63.8%) were found negative (Fig.3.1).

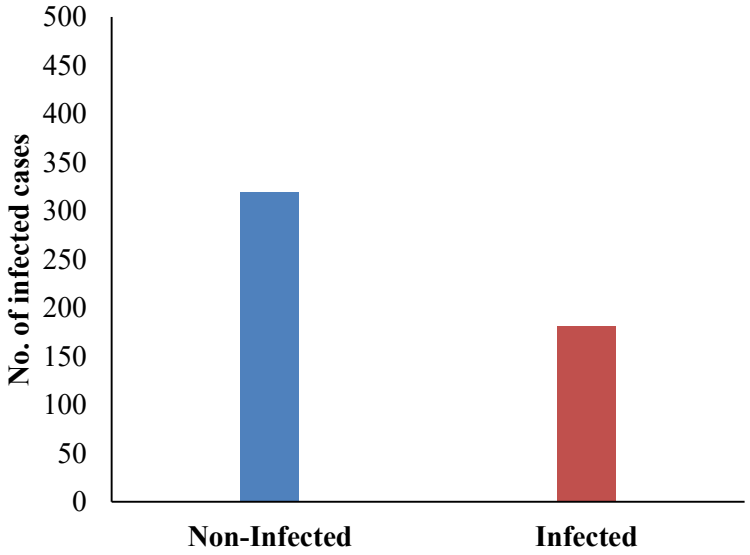

Fig. 3.1: Prevalence of Malaria among participants from district Rawalpindi.

3.1 Gender-wise distribution of malaria

The gender-wise distribution of malarial parasites in examined population is represented in Table 3.1. Among 227 examined males, 85 (46.8%) were found to be infected with malarial and 142 (44.5%) males were found non-infected. In the case of females out of 273 examined females, 96 (53.1%) were found malarial positive and 177 (55.4%) were found non-infected (Fig.3.2). The gender was found significant *(χ^2 = 6.02, P 0.049)* associated with malaria.

Table 3.1: Relationship between malarial infection and gender.

Gender	Infected	Non-Infected	Total
Male	85 (46.9%)	142 (44.5%)	227 (45.4%)
Female	96 (53.1%)	177 (55.4%)	273 (54.6%)
Total	181 (36.2%)	319 (63.8%)	500
Chi-square test = 6.02			***P* value = 0.049**

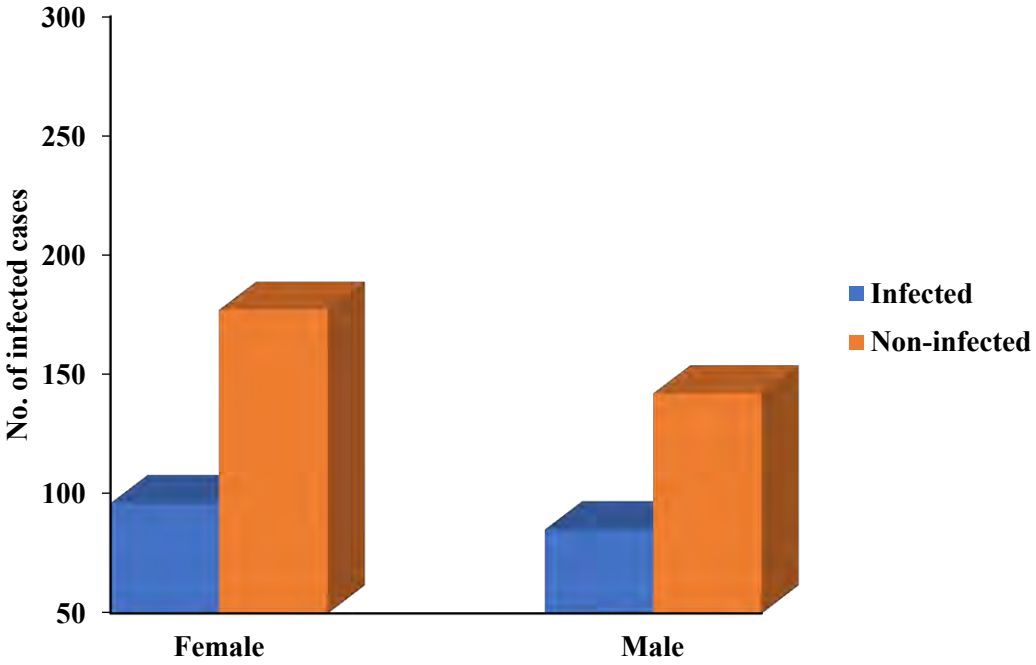

Fig. 3.2: Prevalence of malaria infection on the basis of gender.

3.2 Age-wise distribution of Malaria

The prevalence rate was also measured in different age groups as shown in Table 3.2. The highest infection rate was found within the age ranged between 21 to 40 years (16.6 %) followed by 41 to 60 years (8.6%) and 11-20 young (4.4%), whereas the low number of infected cases were between 1-10 years children (3.4%) (Fig.3.3). The result showed that age association with infection of malaria is statistically significant *(χ^2 = 201, P 0.005).*

Table3.2: Relationship of malarial infection among different age groups.

Age (Years)	Infected	Non-Infected
1-10	17(3.4%)	62(12.4%)
11-20	22(4.4%)	41(8.2%)
21-30	42(8.4%)	69(13.8%)
31-40	41(8.2%	47(9.4%)
41-50	21(4.2%)	34(6.8%)
51-60	22(4.4%)	33(6.6%)
60-70	11(2.2%)	18(3.6%)
71-80	4(0.8%)	10(%)
80-90	1(0.2%)	5(1%)
Chi Square= 201		***P* value= 0.005**

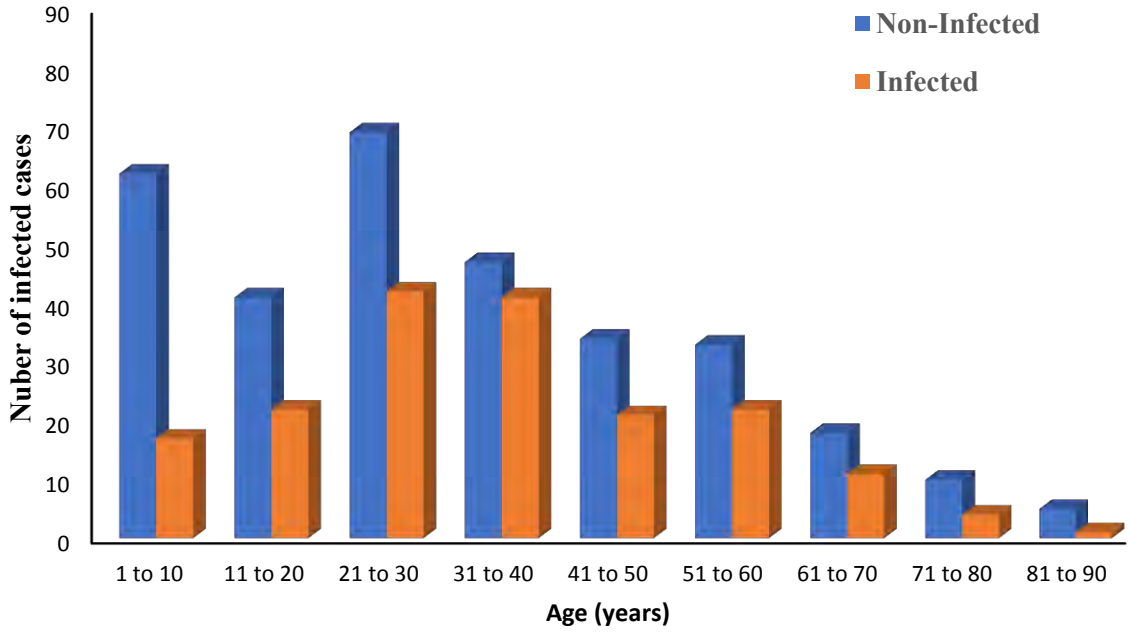

Fig. *3.3*: Prevalence of Malaria infection on the basis of different age groups

3.3 Hematological Perimeters

Hematological features of infected and non-infected males (Table 3.3) and females (Table 3.4) were compared. The results showed red blood cells count did not vary significantly ($P \geq 0.05$) between infected and noninfected male and females. However, a significant ($P \leq 0.05$) reduction in the hemoglobin and platelet count was observed in infected participants. Significant ($P \leq 0.05$) increase in the neutrophiles of infected male was observed, while a significant ($P \leq 0.05$) decrease in lymphocytes, monocytes, eosinophil, were observed in infected participants as compared to uninfected.

Table 3.3: Hematological parameters comparison of infected and non-infected male participants.

Blood parameters	Infected Males (n=85)	Non-infected Males (n=142)	Reference ranges	P-Value
	Mean ±SE	Mean ±SE		
TLC/L	8.33±0.30	9.19±0.65	4.0-10.0X10^9	0. 235
R.B.C/L	4.62±0.07	4.54±0.07	4.50-6.50X10^{12}/L	0.448
Hemoglobin/dL	12.98±0.22	11.86±0.23	13.0-17.0g/dL	0.001*
PCV/L	0.42±0.006	0.36±0.26	0.40-0.54I/L	0.185
MCV/fL	82.76±0.82	85.39±4.92	76.0-96.0 fL	0.599
MCH /pg	28.01±0.33	26.97±0.30	27.0-32.0pg	0.023*
MCHC/dL	33.71±0.15	32.10±0.39	31.5-34.5g/dl	0.0001**
Platelet Count/L	190.40±0.61	270.47±10.70	150-400X10^{12}	0.0001**
Neutrophil %	67.28±1.52	58.11±1.83	40-80%	0.0001**
Lymphocytes %	25.60±1.39	31.52±2.18	20-40%	0.0001**
Monocytes %	4.56±0.24	6.49±0.20	2-10%	0.0001**
Eosinophil %	2.43±0.15	3.02±0.11	1-6%	0.03*

*P≤ 0.05 statistically significant
** P≤0.001 statistically highly significant.

Table 3.4: Hematological parameters comparison of infected and non-infected female participants.

Blood parameters	Infected Females (n=97)	Non-infected Females (n=177)	Reference ranges	P value
	Mean ±SE	Mean ±SE		
TLC/L	8.35±0.29	8.97±0.58	4.0-10.0X10^{9}	0.345
R.B.C/L	4.44±0.05	4.39±0.04	3.80-5.80X10^^{12}/L	0.561
Hemoglobin/dL	11.97±0.22	11.43±0.15	12.0-15.0g/dL	0.045*
PCV/L	0.38±0.005	0.34±0.00	0.37-0.47I/L	0.296
MCV/fL	81.81±0.70	79.26±0.79	76.0-96.0FL	0.017*
MCH /pg	27.38±0.30	26.97±1.34	27.0-32.0pg	0.770
MCHC/dL	33.31±0.15	31.80±0.32	31.5-34.5g/dL	0.0001**
Platelet Count/L	194.50±5.47	270.06±8.00	150-400X10^{9}	0.0001**
Neutrophil %	66.65±1.39	63.85±1.13	40-80%	0.121
Lymphocytes %	26.69±1.25	29.94±1.54	20-40%	0.0001**
Monocytes %	4.44±0.20	6.14±0.21	2-10%	0.0001**
Eosinophil %	2.46±0.16	3.71±0.13	1-6%	0.0001**

*P≤ 0.05 statistically significant

** P≤0.001 statistically highly significant.

3.3.1 RBCs

Red blood cell count was recorded for malarial infected male patients with mean and standard error values of 4.62±0.07, while values in infected female were 4.44 ±0.05. The difference was not statistically significant (P≥0.05) (Fig. 3.4).

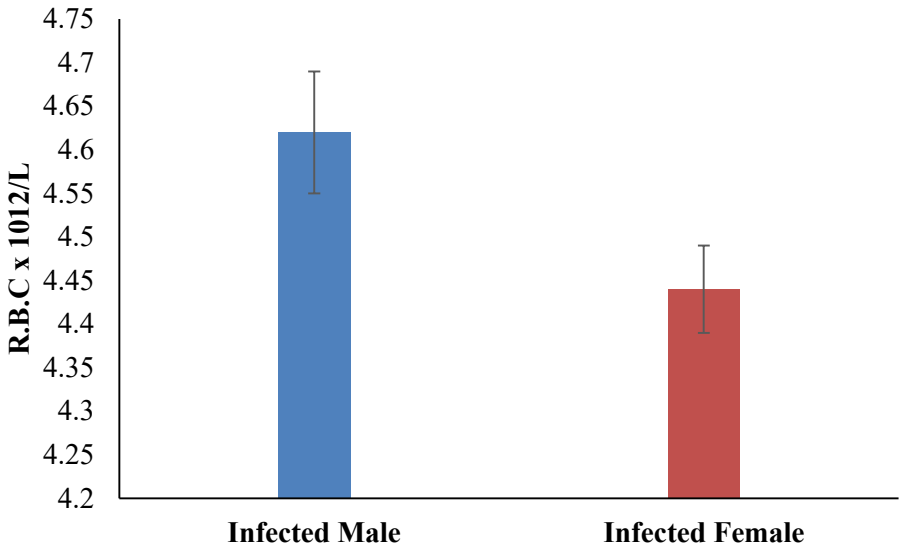

Figure 3.4: RBC count of malarial infected males and females.

3.3.2 Hemoglobin Level

Hemoglobin level for malarial infected patients was calculated with mean and standard error values of males were 12.98±0.22, and in infected female were11.97±0.22. The difference was found significant (p≤0.05) as compared to uninfected males and females (Fig.3.5).

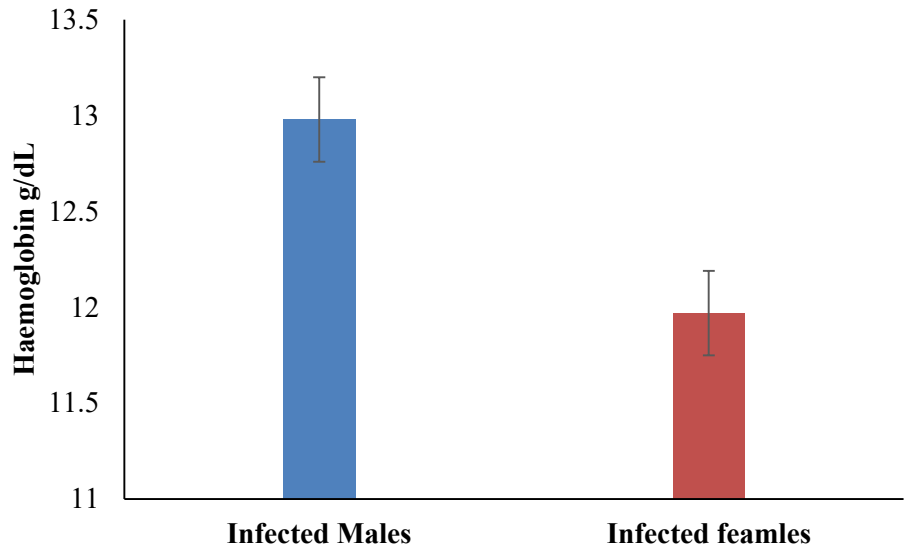

Fig. 3.5: Hemoglobin level of malarial infected males and females.

3.3.3 Mean Corpuscular Volume (MCV)

Mean corpuscular volume (MCV) of red blood cells was observed the mean and standard error values of MCV for malarial infected Male patients were 82.76±0.82. For Female infected patients the calculated values mean and SE were 81.81±0.70. However, the difference in MCV values of malarial infected and non-infected was not statistically significant (P≥0.05) (Fig.3.6).

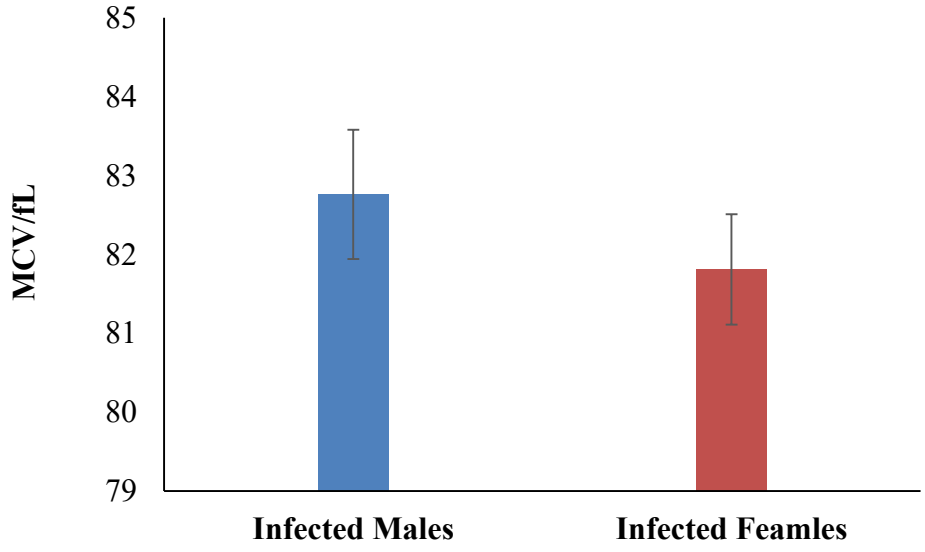

Fig. 3.6: Comparison of MCV of malarial infected Males and Females participants.

3.3.4 Packed cell volume (PCV)

Packed cell volume (PCV) of malarial infected male patient mean and standard error values were 0.42±0.006, while for infected females were calculated as 0.38±0.005. However, the difference in PCV values of infected males and females were not found significant (P≥0.05) (Fig.3.7).

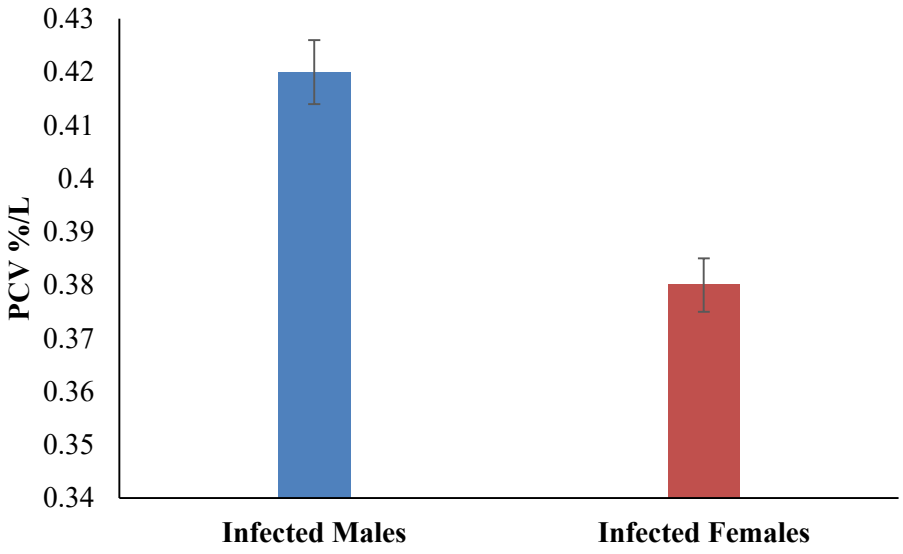

Fig. 3.7: PCV values of malarial infected males and females participants.

3.3.5 Mean corpuscular hemoglobin (MCH)

Mean corpuscular hemoglobin (MCH) values mean and standard error for malarial infected male patients were 28.01±0.33, while in infected females were 27.38±0.30 (Fig. 3.8).

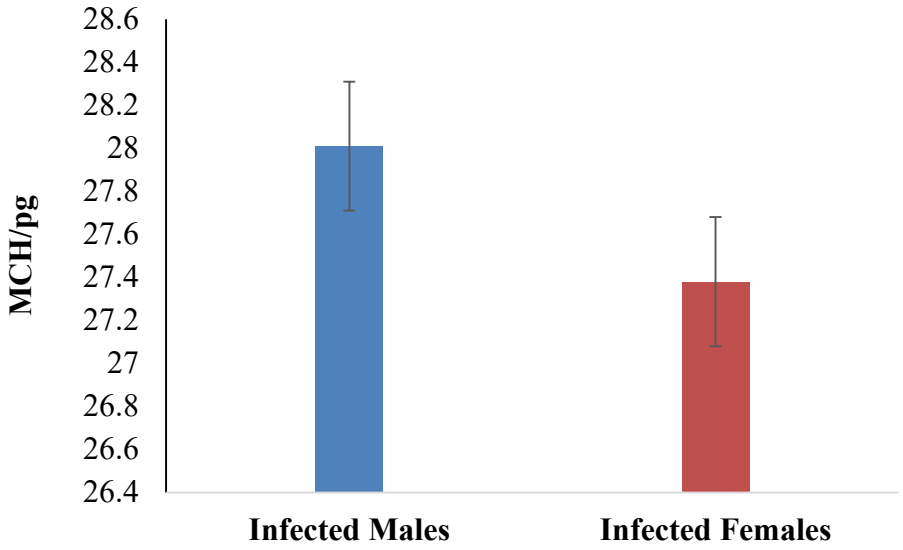

Fig.3.8: MCH values of malarial infected Male and Female infected participants.

3.3.6 Mean corpuscular hemoglobin concentration (MCHC)

Mean corpuscular hemoglobin concentration (MCHC) mean and standard error values were 33.71±0.15 for malarial infected male patients, while for infected female participants was 33.31±0.15 (Fig.3.9). The difference in MCHC values of infected patients was found significant (P= 0.001) as compared to non-infected patients.

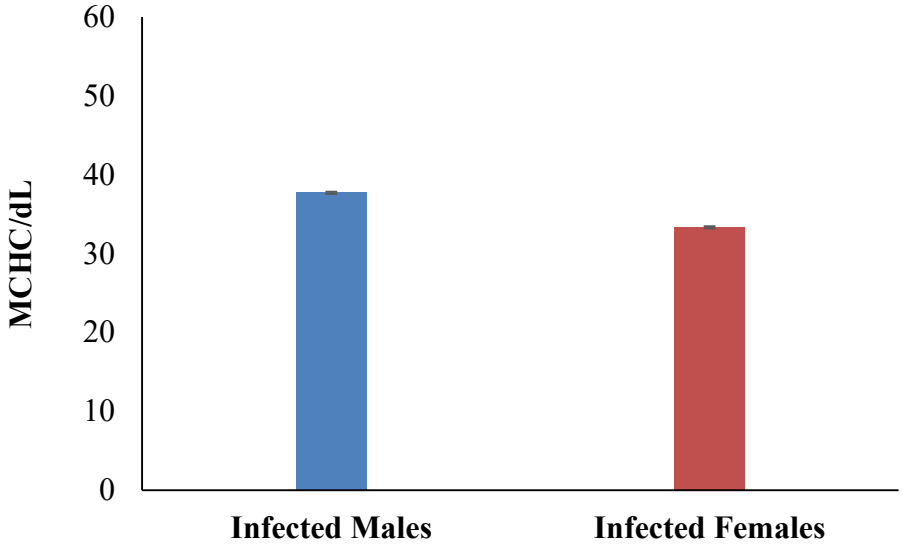

Fig. 3.9: MCHC values of malarial infected Male and infected female participants.

3.3.7 Platelet count

Platelet count mean and standard error values of malarial infected male patients were 190.40±0.61, and for female infected participants were 194.50±5.47. The platelet count was significantly (P= 0.0001) reduced in malarial infected participants (Fig.3.10).

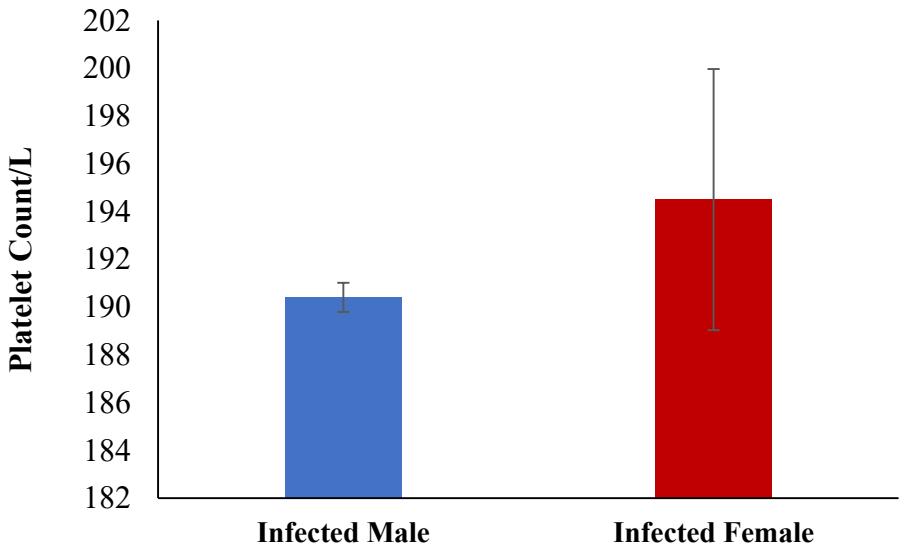

Fig. 3.10. Platelet count of malarial infected Male and female infected participants.

3.3.8 Neutrophils

Neutrophil % mean and standard error values for malarial-infected male patients were 67.28±1.52, while for female infected were 66.65±1.39 (Fig.3.11). However, the difference was found not significant (P≥0.05) in females patients compared to uninfected females.

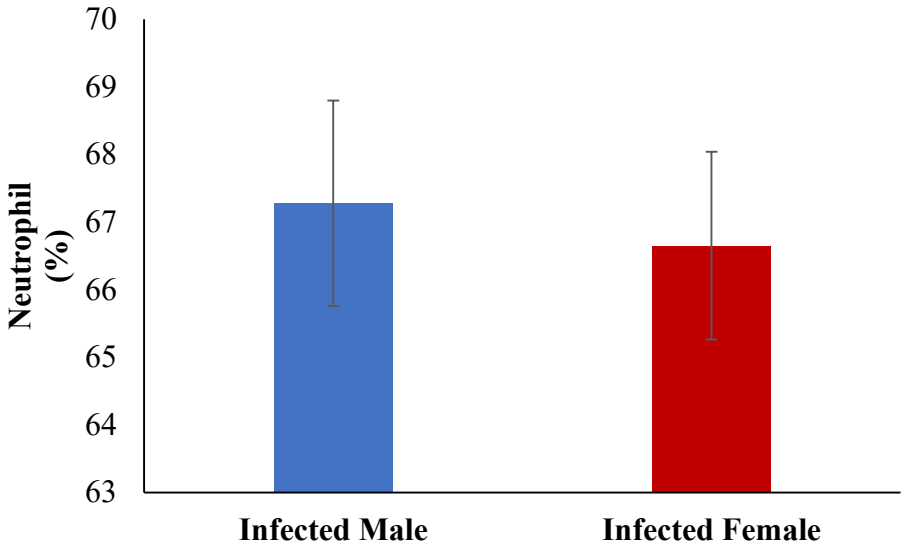

Fig. 3.11. Neutrophil percentage of malarial infected male and female infected participants.

3.3.9 Lymphocytes

Lymphocytes percentage values mean and standard error for malarial-infected male patients were 25.60±1.39, and the mean value for female infected subjects were 26.69±1.25 (Fig. 3.12). This difference was statistically significant (P=0.0001) when compared with uninfected patients.

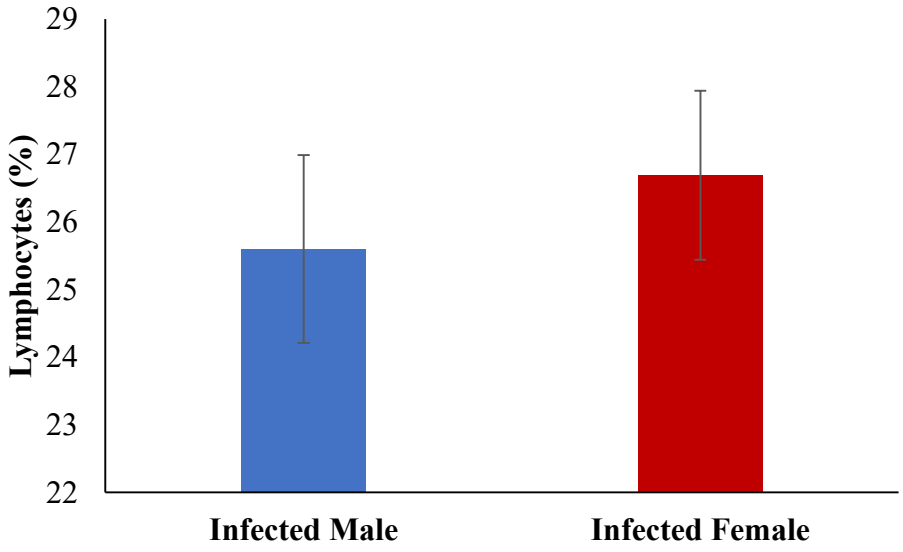

Fig.3.12: Lymphocytes (%) values of malarial infected male and female participants.

3.3.10 Monocytes

Monocyte percentage values mean and standard error for malarial-infected male patients were 4.56±0.24, while for infected females were 4.44±0.20 as shown in Fig. 3.13. However, the difference was significant (P=0.0001) as compared to uninfected patients.

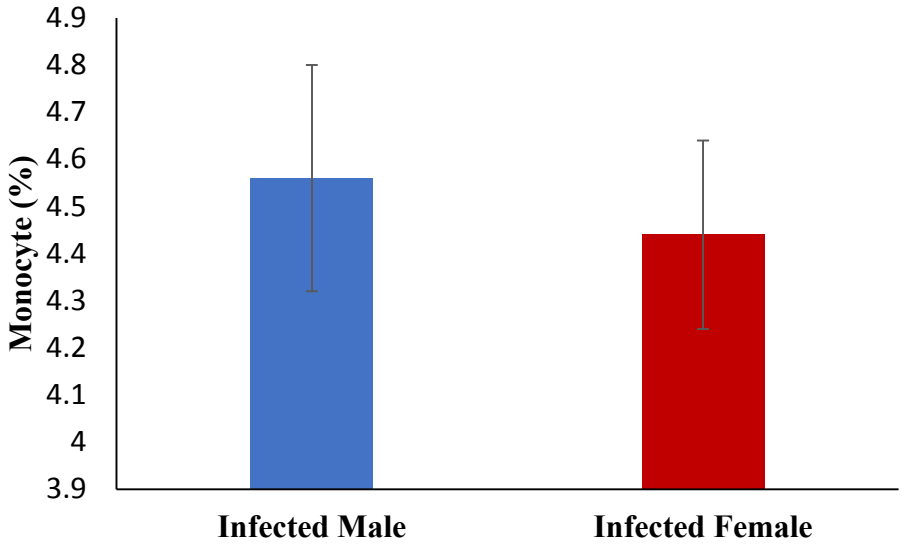

Fig.3.13: Monocytes (%) values of malarial infected male and female patients.

3.3.11 Eosinophil

Eosinophil mean and standard error values of malarial-infected patients were 2.43±0.15, while for infected females were 2.46±0.16 as shown in figure 3.14. However, the difference was significant (P≤0.05).

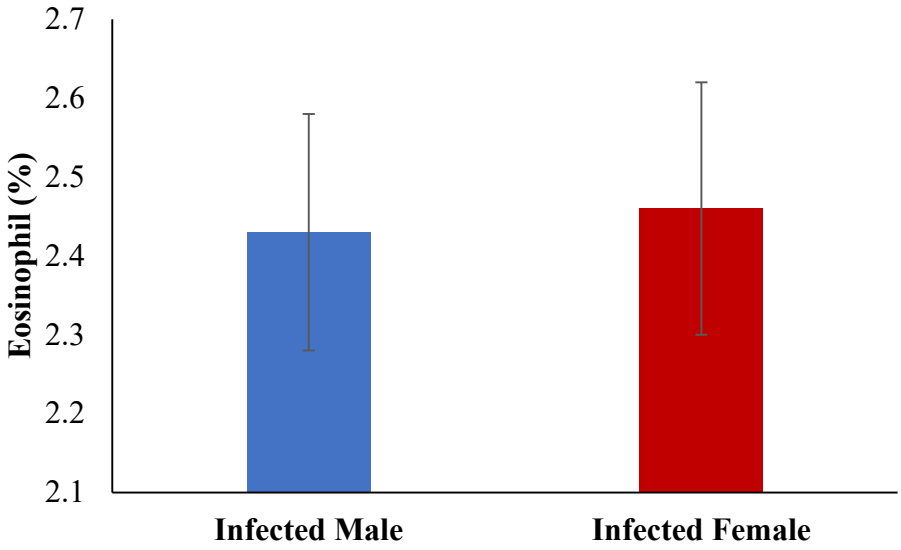

Fig. 3.14: Eosinophil (%) values of malarial infected male and female patients.

3.3.12 Total leucocytes count

Total leucocytes count (TLC) mean and standard error values for malarial infected male patients were 8.33±0.30, and the mean value for infected females were 8.35±0.29 as shown in the Fig.3.15. However, the difference was not significant (P≥0.05).

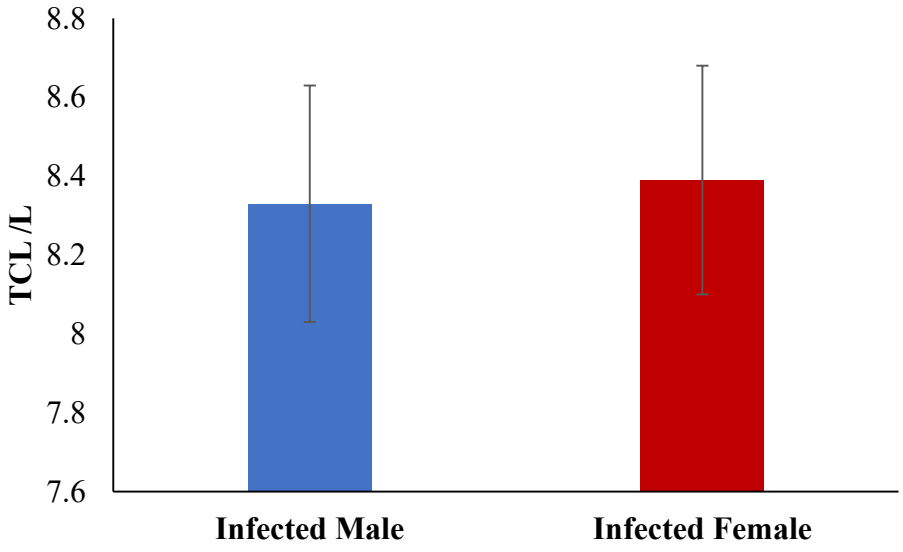

Fig.3.15: TLC/L values of malarial infected male and female patients.

DISCUSSION

Hematological changes associated with malaria infection are well recognized, but specific changes may vary with the level of malaria, background hemoglobinopathy, nutritional status, demographic factors and malaria immunity (Abro *et al*., 2008). Accurate evaluation of malaria infection can also be useful in scaling up control interventions and malaria surveillance in Pakistan (Qureshi *et al*., 2020). Malaria is known to cause several changes in full blood count (FBC) parameters, of which the most prominent are reported to be anemia and thrombocytopenia. Conforming to the literature it was found that the most common problem is hematological abnormalities in many cases of acute malaria infection.

The present study was attempted to determine gender, age, and hematological wise prevalence of malaria. To achieve this goal blood samples were collected from both sexes of 9 age groups in six public healthcare facilities of Rawalpindi from April to September 2022. The Complete Blood Counts were determined, and malarial parasite microscopy was also performed for each patient. CBC was done three-part differential counter (KX-21). White blood cell differential was also done in each case. Overall, 500 people were examined in this study, and 181 (33.2%) people tested positive for malaria . and 313(33.8%) patients who were not affected by the malarial infection.

In the study the gender wise distribution showed that females were infected more 96 (53.1%) as compared to male 85 (46.8%). In contrast to current study other researchers detected more prevalence in males 636 (68.90) population as compared to females 287 (31.09%) (Ibrahim *et al.,* 2014). It may be due the fact that study area males have more chances of bite by mosquitoes, while higher prevalence in females in present study may be more female were examined. Another reason could be due to more exposure to mosquito bites of females due to cleaning of home.

In this study age wise prevalence was found within the age range between 21 to 40 years were found highly affected (16.6 %) followed by 41 to 60 years old (8.6%) and 11-20 young (4.4%), whereas the lowest number of affected were 1–10-year aged

children (3.4%), 60-90 years old adults (3.2%). Similar findings were also reported by Alkadir *et al*. (2020). Another study recorded the highest 19.3% prevalence in age group 5-15 years as compared to other groups (Umaru *et al.,* 2015). Similar trend was also found in another study (Noland *et al*., 2014). The high prevalence among the age group 21-40 years may be due to that people of this age group are concerned with work and outgoing activities.

A comparison of hematological features between the Infected and non-infected groups was done. The hematological parameters differed significantly between infected male and female patients as compared to uninfected were platelets count, hemoglobin, Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), lymphocytes, monocyte and eosinophil. The results were in agreement with previous study (Zeba *et al*., 2014).

The percentage values shows that PCV, MCV and MCH level was higher in infected people as compared to the non-infected people. However, the platelet count was lower in infected people as compared to the non-infected people. The neutrophil level increased significantly in infected people as compared to the non-infected people. However, the lymphocyte, monocytes and eosinophil level decreased significantly in infected people as compared to the non-infected patients. The results are comparable to previous findings (Latif and Jamal, 2015).

CONCLUSION

It is concluded that malaria frequently cause severe changes in blood parameters. Leukopenia and thrombocytopenia more specifically in age groups of 21-40. Anemia and thrombocytopenia were the two most frequent hematological abnormalities seen in this study of malaria infection. The blood changes are so characteristic that the diagnosis of malaria should always be considered in the presence of the above findings.

Future Recommendations

- Furthermore detailed studies should be carried in other districts of Pakistan.
- Awareness programs about malaria should be arranged.

REFRENCES

Abro, A. H., Ustadi, A. M., Younis, N. J., Abdou, A. S., Hamed, D. A., & Saleh, A. A. (2008). Malaria and hematological changes. *Pakistan Journal of Medical Sciences*, *24*(2), 287.

Akhtar, S., Gumashta, R., Mahore, S., & Maimoon, S. (2012). Hematological changes in malaria: a comparative study. *IOSR-JPBS*, *2*(4), 15-9.

Akinosoglou, K. S., Solomou, E. E., & Gogos, C. A. (2012). Malaria: a haematological disease. *Hematology*, *17*(2), 106-114.

Alkadir, S., Gelana, T., & Gebresilassie, A. (2020). A five year trend analysis of malaria prevalence in Guba district, Benishangul-Gumuz regional state, western Ethiopia: a retrospective study. *Tropical Diseases, Travel Medicine and Vaccines*, *6*, 1-7.

Bashawri, L. A., Mandil, A. A., Bahnassy, A. A., & Ahmed, M. A. (2002). Malaria: hematological aspects. *Annals of Saudi medicine*, *22*(5-6), 372-376.

Beeson, J. G., & Brown, G. V. (2002). Pathogenesis of Plasmodium falciparum malaria: the roles of parasite adhesion and antigenic variation. *Cellular and Molecular Life Sciences CMLS*, *59*, 258-271.

Bloland, P. B., Lackritz, E. M., Kazembe, P. N., Were, J. B., Steketee, R., & Campbell, C. C. (1993). Beyond chloroquine: implications of drug resistance for evaluating malaria therapy efficacy and treatment policy in Africa. *Journal of infectious diseases*, *167*(4), 932-937.

Bouma, M. J., Dye, C., & Van der Kaay, H. J. (1996). Falciparum malaria and climate change in the northwest frontier province of Pakistan. *The American journal of tropical medicine and hygiene*, *55*(2), 131-137.

Bray, R. S., & Garnham, P. C. C. (1982). The life-cycle of primate malaria parasites. *British medical bulletin*, *38*(2), 117-122.

Ekvall, H. (2003). Malaria and anemia. *Current opinion in hematology*, *10*(2), 108-114.

Erhart, L. M., Yingyuen, K., Chuanak, N., Buathong, N., Laoboonchai, A., Miller, R. S., & Wongsrichanalai, C. (2004). Hematologic and clinical indices of malaria in a semi-immune

population of western Thailand. *The American journal of tropical medicine and hygiene*, *70*(1), 8-14.

Fairley, M. H. (1947). Sidelights on malaria in man obtained by subinoculation experiments. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *40*(5), 621-76.

Garnham, P. C. C. (1966). Malaria parasites and other Haemosporidia. *Malaria Parasites and other Haemosporidia.*

Garnham, P. C. C., Bray, R. S., Cooper, W., Lainson, R., Awad, F. I., & Williamson, J. (1954). Pre-erythrocytic stages of human malaria: Plasmodium ovale. *British medical journal*, *1*(4856), 257.

Ghanchi, N. K., Shakoor, S., Thaver, A. M., Khan, M. S., Janjua, A., & Beg, M. A. (2016). Current situation and challenges in implementing malaria control strategies in Pakistan. *Critical reviews in microbiology*, *42*(4), 588-593.

Hawking, F., Worms, M. J., & Gammage, K. (1968). 24-and 48-hour cycles of malaria parasites in the blood; their purpose, production and control. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *62*(6), 731-760.

Jairajpuri, Z. S., Rana, S., Hassan, M. J., Nabi, F., & Jetley, S. (2014). An analysis of hematological parameters as a diagnostic test for malaria in patients with acute febrile illness: an institutional experience. *Oman medical journal*, *29*(1), 12.

Kashinkunti, M., & Alevoor, S. (2014). Clinical, hematological and coagulation profile in malaria. *Sch J App Med Sci*, *2*(2B), 584-588.

Khan, M. Q., Zafar, L., Zulfiqar, A., Abbas, A., Salim, H., & Afzal, A. (2022). The Hematological parameters in Malaria and its correlation with parasite Index: A cross-sectional study in Pakistan. *Journal of Haematology and Stem Cell Research*, *2*(1), 31-34.

Khattak, A. A., Venkatesan, M., Nadeem, M. F., Satti, H. S., Yaqoob, A., Strauss, K., & Plowe, C. V. (2013). Prevalence and distribution of human Plasmodium infection in Pakistan. *Malaria journal*, *12*(1), 1-8.

Kimbi, H. K., Sumbele, I. U., Nweboh, M., Anchang-Kimbi, J. K., Lum, E., Nana, Y., & Lehman, L. G. (2013). Malaria and haematologic parameters of pupils at different altitudes along the slope of Mount Cameroon: a cross-sectional study. *Malaria journal*, *12*(1), 1-10.

Kotepui, M., Piwkham, D., PhunPhuech, B., Phiwklam, N., Chupeerach, C., & Duangmano, S. (2015). Effects of malaria parasite density on blood cell parameters. *PloS one*, *10*(3), e0121057.

Latif, I., & Jamal, A. (2015). Hematological changes in complete blood picture in paedriatric patients of malaria caused by plasmodium vivax and falciparum. *Journal of Ayub Medical College Abbottabad*, *27*(2), 351-355.

Malik, A. M., Zaffar, N., Ali, N., Malik, A. M., & Khan, R. (2010). Haematological findings and endemicity of malaria in Gadap region. *J Coll Physicians Surg Pak*, *20*(2), 112-6.

McKenzie, F. E., Prudhomme, W. A., Magill, A. J., Forney, J. R., Permpanich, B., Lucas, C., & Wongsrichanalai, C. (2005). White blood cell counts and malaria. *The Journal of infectious diseases*, *192*(2), 323-330.

Mohamedahmed, K. A., Ahmed, Z. A., Nour, B. Y. M., Abakar, A. D., & Babker, A. M. (2020). Impact of sever Plasmodium falciparum infection on platelets parameters among Sudanese children living in Al-Jazira state.

Mohamedahmed, K. A., Ahmed, Z. A., Nour, B. Y. M., Abakar, A. D., & Babker, A. M. (2020). Impact of sever Plasmodium falciparum infection on platelets parameters among Sudanese children living in Al-Jazira state.

Mukhtar, M. (2006). Killer number one: the fight against malaria: malaria strategy lags behind the global goals, Humanitarian news and analysis a service of the UN Office for the Coordination of Humanitarian Affairs. *Nairobi: IRIN*.

Mulugeta, A., Assefa, A., Eshetie, A., Asmare, B., Birhanie, M., & Gelaw, Y. (2022). Six-year trend analysis of malaria prevalence at University of Gondar Specialized Referral Hospital, Northwest Ethiopia, from 2014 to 2019. *Scientific Reports*, *12*(1), 1411.

Murthy, G. L., Sahay, R. K., Srinivasan, V. R., Upadhaya, A. C., Shantaram, V., & Gayatri, K. (2000). Clinical profile of falciparum malaria in a tertiary care hospital. *Journal of the Indian Medical Association*, *98*(4), 160-2.

Nacher, M., Singhasivanon, P., Silachamroon, U., Treeprasertsuk, S., Tosukhowong, T., Vannaphan, S., & Looareesuwan, S. (2002). Decreased hemoglobin concentrations, hyperparasitemia, and severe malaria are associated with increased Plasmodium falciparum gametocyte carriage. *Journal of Parasitology*, *88*(1), 97-101.

Nsiah, K., Bahaah, B., Afranie, B. O., & Acheampong, E. (2020). Evaluation of red blood cell count as an ancillary index to hemoglobin level in defining the severe falciparum malarial anemia among Ghanaian children in low-resource communities. *Heliyon*, *6*(8), e04605.

Nugraha, N. F., Fauziah, N., Faridah, L., & Hutagalung, J. (2020). The Hemoglobin Levels and Characteristics in Malaria Endemic Region in Eastern Indonesia. *Journal of Health and Medical Sciences*, *3*(1).

Okiring, J., Epstein, A., Namuganga, J. F., Kamya, E. V., Nabende, I., Nassali, M., & Dorsey, G. (2022). Gender difference in the incidence of malaria diagnosed at public health facilities in Uganda. *Malaria Journal*, *21*(1), 1-12.

Pant, C. P., Rishikesh, N., Bang, Y. H., & Smith, A. (1981). Progress in malaria vector control. *Bulletin of the World Health Organization*, *59*(3), 325.

Peprah, S., Ogwang, M. D., Kerchan, P., Reynolds, S. J., Tenge, C. N., Were, P. A.,& Mbulaiteye, S. M. (2020). Mean platelet counts are relatively decreased with malaria but relatively increased with endemic Burkitt Lymphoma in Uganda, Tanzania, and Kenya. *British journal of haematology*, *190*(5), 772-782.

Price, R. N., Simpson, J. A., Nosten, F., Luxemburger, C., Hkirjaroen, L., Ter Kuile, F. E. I. K. O., & White, N. J. (2001). Factors contributing to anemia after uncomplicated falciparum malaria. *The American journal of tropical medicine and hygiene*, *65*(5), 614.

Qureshi, H., Khan, M. I., Ambachew, H., Pan, H. F., & Ye, D. Q. (2020). Baseline survey for malaria prevalence in Khyber Pakhtunkhwa Province, Pakistan. *Eastern Mediterranean Health Journal*, *26*(4), 453-460.

Rojanasthien, S., Surakamolleart, V., Boonpucknavig, S., & Isarangkura, P. (1992). Hematological and coagulation studies in malaria. *Journal of the Medical Association of Thailand= Chotmaihet thangphaet*, *75*, 190-194.

Shortt, H. E., Garnham, P. C. C., Covell, G., & Shute, P. G. (1948). Pre-erythrocytic stage of human malaria, Plasmodium vivax. *British medical journal*, *1*(4550), 547.

Shulman, C. E., Graham, W. J., Jilo, H., Lowe, B. S., New, L., Obiero, J., ... & Marsh, K. (1996). Malaria is an important cause of anaemia in primigravidae: evidence from a district hospital in coastal Kenya. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *90*(5), 535-539.

Sinden, R. E. (1981). Sexual development of malarial parasites in their mosquito vectors. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *75*(1), 171-172.

Singh, P., Singh, S. K., Reddy, V. K., Sharma, S., Chandra, S., & Vijay, P. (2021). A study on association of age, gender, and body mass index with hematological parameters. *Journal of Indian Association of Public Health Dentistry*, *19*(2), 109.

Smith, J. E., & Sinden, R. E. (1982). Studies on the role of host serum in the retention of malarial sporozoites by isolated perfused rat liver. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *76*(1), 45-47.

Sowunmi, A., Fatunmbi, B., Akano, K., Wewe, O. A., Agomo, C., Finomo, F., & Useh, F. (2017). Factors contributing to anaemia after uncomplicated falciparum malaria in under five year-old Nigerian children ten years following adoption of artemisinin-based combination therapies as first-line antimalarials. *BMC infectious diseases*, *17*, 1-18.

Surve, K. M., Kulkarni, A. S., Rathod, S. G., & Bindu, R. S. (2017). Study of haematological parameters in malaria. *Int J Res Med Sci*, *5*(6), 2552-2557.

Tangpukdee, N., Krudsood, S., Kano, S., & Wilairatana, P. (2012). Falciparum malaria parasitemia index for predicting severe malaria. *International journal of laboratory hematology*, *34*(3), 320-327.

Verhave, J. P., Meuwissen, J. T., & Golenser, J. (1980). The dual role of macrophages in the sporozoite-induced malaria infection. A hypothesis. *International journal of nuclear medicine and biology*, *7*(2), 149-156.

World Health Organization. (2022). *World malaria report 2022*. World Health Organization.

Yeda, R., Okudo, C., Owiti, E., Biwot, G., Momanyi, C., Korir, W., & Andagalu, B. (2022). Burden of malaria infection among individuals of varied blood groups in Kenya. *Malaria Journal*, *21*(1), 1-7.

Zeb, J., Khan, M. S., Ullah, N., Ullah, H., Nabi, G., & Aziz, T. (2015). Epidemiology of plasmodium species and prevalence of malaria on the basis of Age, Sex, Area, seasonality and clinical manifestation in the population of district lower Dir, Khyber Pakhtunkhwa, Pakistan. *World Journal of Zoology*, *10*(2), 147-152.

Zeeba Shamim, J., Safia, R., Mohd Jaseem, H., Farhat, N., & Sujata, J. (2014). < An> analysis of hematological parameters as a diagnostic test for malaria in patients with acute febrile illness: an institutional experience.