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FREQUENCY OF MALARIA SPECIES IN LAHORE AND ITS RELATION WITH HEMATOLOGICAL CHANGES

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ABSTRACT

Objective: To determine the incidence of malaria species in our area and to evaluate degree of variation of hematological abnormalities. **Study Design:** Descriptive study **Settings:** Farooq Hospital west wood branch affiliated with Akhter Saeed Medical and Dental College **Period:** August 2015 to July 2017. **Material and Methods:** Total 320 malaria positive patients fulfilling our inclusion criteria were enrolled in this study. Patients with known thrombocytopenia history, chronic liver disease and dengue co-infection were excluded. Complete blood counts of patient were performed on Sysmex KX-21 analyzer. Thick and thin blood film examinations were performed for malarial parasite confirmation and specie identification. Patients were divided in 5 groups on basis of age including $\leq$15, 16-30, 31-45, 46-60 and $\geq$61 years. Thrombocytopenia was categorized as mild, moderate and severe if platelets count was 51-150 x 10^9/l, 20-50 x 10^9/l and <20 x 10^9/l respectively. Anemia was categorized as mild, moderate and severe if Hemoglobin level was above 10 g/dl but below normal level, 8.0-10 g/dl and <8.0 g/dl respectively. Data was analyzed using SPSS version 24.0. **Results:** Out of 320 enrolled malaria positive patients, 278 (86.9%) were infected with *Plasmodium vivax* and 42 (13.1%) were positive for *Plasmodium falciparum.* Overall 258 (80.6%) patients presented thrombocytopenia, 55 (17.2%) patients presented leukopenia and 190 (59.4%) patients presented anemia. Anemia was more frequently observed in two age groups including $\leq$15years and 16-30 years whereas $\leq$15 years age group presented significantly high incidence of microcytic hypochromic anemia (*p-value 0.000)*. Significant variations in results of hematological findings were observed in patients infected with different malarial species. Finding of anemia was more common in *P. falciparum* malaria whereas frequency of severe and moderate thrombocytopenia was also significantly highin *Falciparum* infected patients (*p-value* 0.000). Statistically significant variation in relative and absolute neutrophils and lymphocytes count was observed. **Conclusion:** In our setup *P. vivax* infection is more prevalent than *P. falciparum.* Malaria causes significant variation in hematological findings. Thrombocytopenia is commonly observed especially in patients infected with *P. falciparum*. Addition of absolute differential leukocyte counts in reporting of complete blood counts can improve sensitivity of results and improvement in diagnosis.

Key words: Malaria, Thrombocytopenia lymphopenia complete blood counts (CBC)

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INTRODUCTION

Malaria has deep roots in history of mankind but still mortality and morbidity due to malaria is a major concern.[1] It is prevalent worldwide with high incidence in tropics and enlisted as one of the biggest killer infection. Almost 250 million cases of malaria are reported globally with approximately one million deaths annually.[2]

Malaria is an endemic disease in Pakistan.[3] It is transmitted via female anopheles mosquito while taking a blood meal, leaving malarial sporozoites in human blood circulation. Malarial parasites primarily infects red blood cells (RBCs), liver and spleen.[1] Many hematological abnormalities are associated with malaria including abnormalities of white blood cells(WBCs), platelets and hemoglobin levels.[4]

Thrombocytopenia is reported as a common finding in malaria patients with incidence ranging between 40 to 85% of cases.[1] Thrombocytopenia serves as a diagnostic and prognostic indicator of malarial infection accompanied with high grade fever.[5] Many mechanisms are involved to cause thrombocytopenia in malaria patients including immunoglobulin G (IgG) mediated immune destruction, destruction by activity of macrophages, increased splenic pool of platelets, raised cytokines level, oxidative stress and platelets clumps formation in circulation.[1,6,7] This thrombocytopenia is reported to enhance complications of malarial infection.[8] Low platelets count (less than 100 x 10^9/l) in malaria is associated with significantly high mortality rate than that of platelets count above 100 x 10^9/l.[9] Recent experiments have demonstrated that platelets are related with immune function against malarial parasite as they bind with parasitized red blood cells (RBCs) and kill parasites present within them.[10]

Malaria is also reported to cause anemia and leukocytopenia as well along with varying degree of lymphopenia.[1,11,12,13] In pediatric patients severe anemia is reported in cases of infection with *P. falciparum*.[3]

Two species of malaria are prevalent in Pakistan including *Plasmodium falciparum* and *Plasmodium vivax.* Malaria caused by *P. vivax* is called benign malaria with moderate to mild thrombocytopenia and less severe symptoms.[14] Whereas malaria caused by *P. falciparum* is called malignant malaria resulting in severe thrombocytopenia along with cerebral malaria, organ failure and even shock leading to death.[2] Recent published data suggest that infection with *P. vivax* may also lead to same symptoms and complications like *P. falciparum* by following same mechanism adopted by *P. falciparum* to cause malignant malaria.[15]

OBJECTIVES

The aim of this study were

- To determine the incidence of malaria species in our setup.
- To assess the occurrence and severity of WBCs, RBCs and platelets abnormalities in malaria positive patients and also
- To determine the effect of malarial specie on patient's hematological findings.

MATERIAL AND METHODS

Study design

A descriptive cross-sectional study.

Setting

Study was conducted in Farooq hospital west wood branch Lahore affiliated with Akhter Saeed Medical and Dental College (AMDC) Lahore.

Study duration

From August 2015 to July 2017.

Sample Size

Total 320 malaria positive patients were included in this study.

Sample Technique

Convenient Sampling Technique

Exclusion criteria

Patients with previously known history of thromboc-ytopenia, chronic liver disease and those with co-infection with dengue were excluded from this study.

DATA COLLECTION

Demographic data and complete blood count (CBC) results of patients were collected using previously designed proforma. Thick and thin blood smears were examined for the diagnosis and specie identification of malarial parasite. Complete blood counts (CBC) were performed on Sysmex KX-21 semi-automated hematology

analyzer. Patients were divided into 5 groups on the basis of their age. These groups included less than or equal to 15($\leq$15) years, 16-30 years, 31-45 years, 46-60 years and more than or equal($\geq$61) to 61 years of age. Total leukocyte count (TLC) 4-11 x 10^9/liter was considered as normal. Hemoglobin (Hb) level above 13.5 g/dl in male and above 12.0 g/dl in female were considered normal.

Hemoglobin level 10.0-13.5 g/dl in male and 10.0-12.0 g/dl in female were considered as mild anemia, 8.0-10.0 g/dl in both gender was considered as moderate anemia and less than 8.0 g/dl was considered as severe anemia. Platelets count below than 150 x 10^9/liter was considered as thrombocytopenia.

Thrombocytopenia was categorized in three categories. Platelets count between 50-150 x 10^9/liter, 20-50 x 10^9/liter and less than 20 x 10^9/liter were labelled as mild, moderate and severe thrombocytopenia respectively. Relative and absolute counts of neutrophils and lymphocyte were performed. Relative neutrophil count between 40-75% and absolute neutrophil count between 2.5-7.5 x 10^9/liter were considered as normal. Relative and absolute lymphocyte count between 20-45% and 1.5-3.5 x 10^9/liter respectively were considered normal.

Data Analysis

Data was analyzed using SPSS version 24.0. Categorical variables were expressed as frequencies and percentages whereas continuous variables were expressed as Mean $\pm$ SD. Chi-square test Pearson correlation and independent samples t-test were used to analyze data. *P-value*$\leq$ 0.05 was considered as statistically significant.

RESULTS

Total 320 patients were included in this study out of these 216 (67.5%) were male and 104 (32.5%) were females. Distribution of patients according to their age groupsand frequency of anemia and its types is presented in table. 1.

The results of study indicated that finding of anemia was more pronounced in age group $\leq$15 and 16-30 years. Significant correlation was found between age of patients and occurrence of microcytic hypochromic anemia (*p-value=0.000*). Incidence of microcytic hypochromic anemia decreases with increase in age and it was most common in age group $\leq$15 years.

The results of this study showed that infection with *Plasmodium vivax was* more common cause of malaria in these patients as 278 (86.9%) patients were positive for *P. vivax* and only 42 (13.1%) patients were infected with *P. falciparum*.

The comparison of variation in hematological findings of patients observed in both malarial species is presented.

The results of this study also indicated that finding of anemia was more pronounced in patients infected with *P. falciparum*when compared with *P. vivax.* Out of 42 *P. falciparum* positives cases 29 (69.0%) patients showed anemia out of which severe anemia was observed in 4 (9.5%) patients whereas 6 (14.3%) and 19 (45.2%) patients showed moderate and mild anemia respectively. Contrary to this out of 278 *P. vivax* positive patients 161 (58.0%) presented anemia out of which 10 (3.6%) presented severe anemia and 33 (11.9%) and 118 (42.4%) showed moderate and mild anemia.

Thrombocytopenia was frequently found in these patients. Total 258 (80.6%) patients presented thrombocytopenia out of which 5 (1.9%) showed severe thrombocytopenia, 44 (17%) presented moderate thrombocytopenia and 209 (81.1%) patients showed mild thrombocytopenia.

The results of study showed significant difference in platelets levels of patients infected with *P. falciparum* and *P. vivax.* Severe thrombocytopenia was more common finding in *P. falciparum.* On the other hand mild thrombocytopenia was more pronounced in patients infected with *P. vivax.* Frequency of severe, moderate andmild thrombocytopenia in *P. falciparum* and *P. vivax* infection is presented.

Out of 320 malaria positive patients enrolled in this study, most of patients showed normal TLC results. Finding of leukopenia was observed in 55 (17.2%) patients whereas leukocytosis was observed in 13 (4.1%) patients. The results of this study indicated that the commonly used relative neutrophils and lymphocytes counts may not be the true predictor of patient's situation as significant variation in results was observed when we compared the relative and absolute counts of these two in malaria positive patients.

Table No. 01: Distribution of patients in age groups and frequency of anemia and its types

			Type of anemia (severity)			Type of anemia (Morphological)		
Age group (years)	Total patients	Anemic patients	Mild	Moderate	Severe	Micro-cytic Hypoc-hromic	Normo-cytic Normoc-hromic	Macro-cytic
<15	77 (24.1%)	68 (88.3%)	38 (55.9%)	23 (33.8%)	7 (10.3%)	47 (69.1%)	21 (30.9%)	-
16-30	156 (48.7%)	77 (49.3%)	61 (79.2%)	11 (14.3%)	5 (6.5%)	36 (46.7%)	41 (53.3%)	-
31-45	48 (15.0%)	23 (47.9%)	19 (82.6%)	4 (17.4%)	-	6 (26.1%)	17 (73.9%)	-
46-60	32 (10.0%)	19 (59.3%)	16 (84.2%)	1 (5.3%)	2 (10.5%)	6 (31.6%)	12 (63.1%)	1 (5.3%)
>61	7 (2.2%)	3 (42.8%)	3 (100%)	-	-	1 (33.3%)	2 (66.7%)	-
Total	320 (100%)	190 (59.4%)	137 (72.1%)	39 (20.5%)	14 (7.4%)	96 (50.5%)	93 (49%)	1 (0.5%)

Table No. 02: Comparison of CBC results in *P. falciparum* and *P. vivax* infected patients

	P. Falciparum		*P. Vivax*		*P value*
	Range	Mean+SD	Range	Mean+SD	
TLC (x10^9/1)	0.80-16.70	5.59±2.74	1.30-21.10	6.13±2.59	0.209
Hemoglobin (g/dl)	6.8-16.7	11.8±2.4	4.9-16.7	12.2±2.1	0.261
Hematocrit (%)	21.50	35.7±7.4	15-49	36.1±5.9	0.677
MCV (fl)	53.92	80.4±7.4	54-104	80.7±7.1	0.779
MCH (pg)	15.31	26.5±2.9	17-35	26.7±3.0	0.580
RBCs count x 10^{12}/1	2.20-5.70	4.32±0.85	1.90-6.50	4.46±0.69	0.251
Platelet count (x 10^9/1)	10-413	60.5±62.2	35-455	118.4±62.0	0.000*
Relative Neutrophils-count (%)	20-90	66.0±15.1	20-90	68.5±14.48	0.304
Absolute Neutro-phil count (x 10^9/1)	0.16-15.03	3.79±2.31	0.80-14.70	4.22±2.09	0.215
Relative Lympho-cyte count (%)	5-75	26.8±14.9	5-300	24.9±21.37	0.596
Absolute Lympho-cyte count (x 10^9/1)	0.47-6.45	1.40±1.1	0.23-12.23	1.43±1.17	0.918

Table No. 03: Frequency of severe, moderate and mild thrombocytopenia in patients

	Plasmodium falciparum		*Plasmodium vivax*		
	Frequency	Percentage%	Frequency	Percentage%	*p. value*
Severe thrombocytopenia	5	11.9	00	00	0.000*
Moderate thrombocytopenia	21	50.0	23	8.2	0.000*
Mild thrombocytopenia	15	35.7	194	69.8	0.000*
Normal count	1	2.4	61	21.9	0.000*

Table No. 04: Frequency of Differential Leukocyte Counts (DLC) of patients

Investigation	Frequency	Percentage	Mean±SD	Range	P value
Relative Neutropenia (%)	10	3.1	25.2±4.96	20-35	0.00*
Absolute Neutropenia (x10^9/l)	61	19.1	1.88±0.50	0.16-2.49	
Relative Neutrophilia (%)	110	34.4	82.17±4.30	76-90	0.00*
Absolute Neutrophilia (x10^9/l)	21	6.6	9.59±2.07	7.57-15.03	
Relative Lymphopenia (%)	141	44.1	12.8±3.97	5-30	0.00*
Absolute Lymphopenia (x10^9/l)	211	65.9	0.87±0.32	0.23-1.47	
Relative Lymphocytosis (%)	21	6.6	56.62±9.90	46-75	0.00*
Absolute Lymphocytosis (x10^9/l)	12	3.8	4.55±1.47	3.5-7.78	

Comparison of relative and absolute count of neutrophil and lymphocyte with frequency, Mean ± SD, and range is presented.

DISCUSSION

Malaria remains as one of the most common cause of acute febrile illness in Pakistan. Many hematological changes are reported in malarial infection[16].Malarial parasite affects all cellular components of the blood. Multiple factors are involved in the severity of malaria infection including increased intra and extracellular hemolysis, structural malformation of infected RBCs, markedly decreased production of platelets accompanied with short life span in circulation and augmented splenic pool results in anemia and thrombocytopenia. Circulation of infected RBCs to deep vessels of major organs results in cerebral malaria, renal failure, and hepatic dysfunction.[2]

The results of this study revealed that males were predominantly affected (67.5%) with malaria as compared with females (32.5%). Same results had been reported previously.[1, 2, 3, 16, 17] These result can be explained by the fact that male have more

outdoor activities as compare to female which makes them easy target for transmission of malarial parasite via mosquito bite. Another important observation of study was that *P. vivax* was leading (86.9%) cause of malaria in our setup whereas only 13.1% patients were found positive for *P. falciparum* infection. Khattak AA *et al* in 2013 conducted a study on incidence of malaria species using polymerase chain reaction (PCR) to identify malaria specie and collected samples from all over Pakistan he also reported high incidence of *P. vivax* in Lahore region of Pakistan.[14]No study focusing Lahore region only has been conducted so far. Contrary to this many studies conducted in other regions of Pakistan had reported markedly high frequency of *P. falciparum* in different regions.[3, 16, 17]

Significance of thrombocytopenia in pathogenesis of malaria infection is well documented now. In present study thrombocytopenia was evident in 80.6% patients. Almost similar results had been reported before in various studies.[1, 3, 16, 19, 20] Contrary to this many studies had reported variation in incidence of thrombocytopenia.[18,21]

In last decade researchers from different regions of world have reported *P. vivax* infection as a cause of severe thrombocytopenia and fatal clinical manifestations. In their opinion different genetic modifications in *P. vivax* has increased its pathogenicity.[2, 15, 22] The focus of these studies was on the fact that like *P. falciparum, P. vivax* infected patients are also showing severe degree of thrombocytopenia and their clinical symptoms are more like malignant malaria rather than classical benign malaria. However present study contradicts with results of these studies in relation of thrombocytopenia. All patients experience severe thrombocytopenia were infected with *P. falciparum* whereas incidence of moderate thrombocytopenia was also significantly high in *P. falciparum* infection and most patients presented with mild thrombocytopenia were infected with *P. vivax.*

About 60% of patients enrolled in this study presented withvarying degree of anemia. Abro AH *et al* had reported almost similar incidence of anemia in malaria positive patients.[19] However morphological type of anemia reported in present study is different from Abro AH. In present study incidence of microcytic hypochromic anemia was high and most of patients experienced microcytic hypochromic anemia belong to age group $\leq$15 years. Further studies of large scale must be done to rule out iron deficiency anemia and thalassemia trait in these patients as these two condition are common in Pakistan and are documented cause of microcytic hypochromic anemia particularly iron deficiency anemia in school going age group. Incidence of anemia reported in our study however disregard with frequency of anemia reported by Tanveer M *et al* where anemia was found only in 39.6% of patients.[16]

In present study 78.8% patients showed normal WBC count, 17.2% showed leukopenia and 4.1% had leukocytosis. Similar results are reported previously.[19] In this study results of absolute and relative differential leukocytes counts were compared and an important finding was observed that results of relative and absolute counts of neutrophils and lymphocytes were not relating with each other. Relative differential leukocyte count is still the most popular reporting pattern in laboratories of Pakistan. Whereas absolute differential leukocyte countis not reported in routine. This variation in results show that commonly used relative parameters are not good indicator of patients condition and pathologists and clinicians need to focus on using absolute countsto improve diagnostic outcome.

Limitation

Patient's clinical symptoms along with management provided and response of patient towards treatment are not followed up in this study. Other laboratory parameters like liver functions test (LFTs) are not included in this study to access effect of malaria on liver.

CONCLUSION

This study exhibits that *P. vivax* infection is more common in our setup than *P. falciparum* infection. The most affected age group is 16-30 years. Malaria infection significantly affect the hematological parameters especially thrombocytopenia, anemia and lymphopenia are the most common hematological findings observed in these patients in our setup. Level of thrombocytopenia was significantly high in patients suffering from falciparum malaria. The observation in this study reveals that absolute differential counts are more accurate indicator of disease condition as compared to relative counts.

Conflict of Interest
None to declare.
Source of Funding
None to declare.

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