ORIGINAL ARTICLE

Haematological Findings and Endemicity of Malaria in Gadap Region

Ansar Mahmood Malik[1], Nusrat Zaffar[2], Nadir Ali[4], Aminah Mahmood Malik[3] and Rubina Khan[1]

ABSTRACT

Objective: To determine the frequency of *Plasmodium (P.) vivax* and *P. falciparum* in cases of acute febrile illness, and the haematological parameters in patients suffering with acute malaria.

Study Design: An observational study.

Place and Duration of Study: Baqai Medical University, Fatima Hospital Laboratory, Karachi, from January 2006 to December 2007.

Methodology: Patients with acute febrile illness were evaluated. Complete blood count and malarial parasite were performed. Descriptive statistics of haematological parameters was computed. Mean and standard deviations (SD) were calculated; p-value was determined using test of proportions on SPSS.

Results: Out of 3344 patients, 392 (11.72%) were proved as suffering from malaria with male to female ratio of 1.36. The age ranged from 1 month to 94 years. One hundred and seventy six (42.6%) had *P. falciparum*, 204 (52%) had *P. vivax*, and 21 (5.4%) had mixed infection. Haemoglobin varied from 1.10 g/dl -17.10 g/dl, (mean 9.83 $\pm$ 3.09 g/dl). TLC ranged from 1.30 x 10^3/ul - 48.50 x 10^3/ul, (mean 6.80 $\pm$ 5.15 x 10^3/ul. The platelet counts ranged from 10.0 x 10^3/ul - 850 x 10^3/ul, (mean 106 $\pm$ 90.98 x 10^3/ul). Thrombocytopenia was observed in 70%, platelet count was significantly lower in patients with mixed infection as compared to patients with *P. falciparum* and *P. vivax* (p=.017).

Conclusion: The frequency of malaria was 11.72%. Thrombocytopenia was the most common finding. In none of the patients platelet counts dropped below the critical levels of 10 x 10^3/µl.

Key words: *Malaria. Thrombocytopenia. Plasmodium vivax. Plasmodium falciparum.*

INTRODUCTION

Malaria is one of the most prevalent human infections worldwide. Exact numbers are unknown but an estimated 300–500 million cases of malaria, and 1.5–2.7 million malaria related deaths occur each year. The majority of cases and almost all deaths are caused by *Plasmodium falciparum (P. falciparum)*, and *Plasmodium vivax (P. vivax). Plasmodium ovale (P. ovale)* and Plasmodium malariae *(P. malariae)* cause less severe disease.[1] Malaria presents with fever chills and rigors, and may mimic other common febrile conditions like enteric, and dengue fevers.

Haematological abnormalities have been observed in patients with malaria; anaemia and thrombocytopenia being the most common.[2] Similar haematological findings are also observed in other common illnesses specially in typhoid and dengue fever, both illnesses are also endemic in Pakistan.

Department of Medicine[1]/Pathology[2]/Dentistry[3], Baqai Medical University, Karachi.
Department of Haematology[4], Combined Military Hospital, Malir Cantt., Karachi.

***Correspondence:** Dr. Ansar Mahmood Malik, 42 Alauddin Road, Lahore Cantt.*
E-mail: ansarmmalik@yahoo.com

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Pakistan being a part of the endemic belt has a high incidence of malaria.[3] Although a few studies report an incidence of malaria up to 5 million persons annually in Pakistan, still there is no accurate data available from all provinces.[4] *Falciparum* and *vivax* malaria are major health problems in Pakistan. In the last decade there has been a six fold increase in *falciparum* malaria, which now comprises 42% of all malaria cases recorded by the National Malaria Control Program. At least 39 districts, mainly from the two southern provinces of Balochistan and Sindh, have been classified as high risk, partly due to the weak public health infrastructure.[5] The available data reveals the coastal city of Karachi, Thatta, Badin, Zhob, Larkana and Nawabshah as holoendemic for malaria with round the year transmission.[4]

This study was conducted to determine the frequency of *P. vivax* and *P. falciparum* in patients presenting with acute febrile illness in the Gaddap Town, Karachi, and also to find out the haematological parameters in patients suffering with acute malaria.

METHODOLOGY

It was an observational study, conducted from January 2006 to December 2007. All patients with acute febrile illness consulting Fatima Hospital, Gaddap Town, were evaluated. Patients with established diagnosis of systemic infections, typhoid fever, dengue fever, and

meningitis were excluded from the study. Two milliliters of venous blood sample was collected from antecubital vein after ensuring aseptic skin preparation. Blood was immediately transferred to a tube containing ethylene diamine tetra-acetic acid (EDTA) anticoagulant. A complete blood count (CBC) and malarial parasite microscopy were performed for each patient. The haematological parameters were carried out on SYSMEX-POCH 100 i haematology analyzer. Thick smears for malarial parasites were prepared by placing a small drop of blood in the centre of the slide and spreading it out with the corner of another slide to cover an area about four times its original size. The smears were allowed to dry for at least 30 minutes at 37°C before staining. The thin smear slides were made and stained with Leishman's stain as described by Bain and Lewis.[6] The slides were examined by qualified pathologists and malarial parasites were identified. Once the presence of parasites was confirmed, a thin film was examined for recognizing the species *P. falciparum* and *P. vivax*. The smears were examined under 40x and 100x objective oil immersion for species recognition. In addition haemoglobin (Hb%), total leukocyte count (TLC), and platelet counts were recorded.

The data was analyzed using the Statistical Package for Social Sciences (SPSS) version 11.0. Descriptive statistics of haemoglobin, total leukocyte count, platelet count and other haematological parameters were computed. Mean and standard deviations (SD) were calculated and compared for quantitative variables and proportions were determined and compared for categorical variables. P-values < 0.05 were considered statistically significant.

RESULTS

A total number of 3344 patients with acute febrile illness without established diagnosis were received. Three hundred and ninety-two (11.72%) patients revealed malarial parasite on blood film examination. Two hundred and twenty-six (58%) were males and 166 (42%) were females. The mean age of the patients was 21.7±16.46 years with a minimum 1 month and a maximum 94 years. One hundred and sixty (40.8%) of the malaria positive patients were children with age less than 15 years, while 232 (59.1%) were above 15 years of age. One hundred and sixty-seven (42.6%) of the patients had *P. falciparum*, 204 (52%) had *P. vivax* and 21 (5.4%) had a mixed infection of both *P. falciparum* and *P. vivax*. In 160 patients of paediatric age group, *P. falciparum* was detected in 62 (38.8%), *P. vivax* in 86 (53.8%) and mixed infection was detected in 12 (7.5%).

Haemoglobin (Hb) of the patients ranged from 1.10 g/dl to 17.10 g/dl with a mean value of 9.83 ± 3.09 g/dl. TLC of the patients ranged from 1.30 x 10^3/ul to 48.50 x 10^3/ul (mean 6.80 ± 5.15 x 10^3/ul. The distribution of Hb% and TLC in all three groups is shown in Table I.

The platelet counts of the patients ranged from 10.0 x 10^3/ul to 850 x 10^3/ul with a mean value of 106.7 ± 90.98 x10^3/ul. Thrombocytopenia (count < 150 x 10^3/ul) was observed in 70% of the patients. The platelet count in *P. vivax*, *P. falciparum* and mixed infections has been compared in Table II. There was no statistically significant difference between the total leukocyte counts among *falciparum*, *vivax* and mixed infection patients (p=0.07). Patients with mixed infection had a significantly lower platelet count (mean 65.4 x 10^3/ul) as compared to the patients with P. falciparum (mean 95.3 x 10^3/ul), and *P vivax* (mean 119.1 x 10^3/u, p=.017). Platelet counts of all three groups have been compared in Figure 1.

Table I: Haemoglobin and total leukocyte count in malaria in all age groups.

Parameter	Range	Number of patients	Percentage of patients
Hb (g/dl)	< 3.0	5	1.34
	3.1 – 6.0	45	12.1
	6.1 – 9.0	87	23.4
	9.1 – 12.0	140	37.7
	12.1 and >	94	25.3
TLC (x 10^3/ul)	< 2.0	8	2.17
	2.1 - 4.0	68	18.47
	4.1 – 8.0	213	57.8
	8.1 – 11.0	46	12.5
	11.1 – 15.0	17	4.6
	15.1 – 20.0	7	1.90
	20.1 and >	9	2.44

Hb: Haemoglobin; TLC: Total leukocyte count.

Table II: Platelet counts in patients of all age groups.

Platelet counts (x 10^3/ul)	*P. vivax* n=188	*P. falciparum* n=157	Mixed infestation n=19
< 30.0	30 (15.9%)	39 (24.8%)	4 (21%)
31-60	25 (13.29%)	36 (22.9%)	6 (31.5%)
61-90	27 (14.36%)	27 (17.19%)	6 (31.5%)
91-120	15 (7.97%)	10 (6.3%)	1 (5.26%)
121-150	20 (10.63%)	9 (5.7%)	1 (5.26%)
> 150	71 (37.76%)	36 (22.9%)	1 (5.26%)

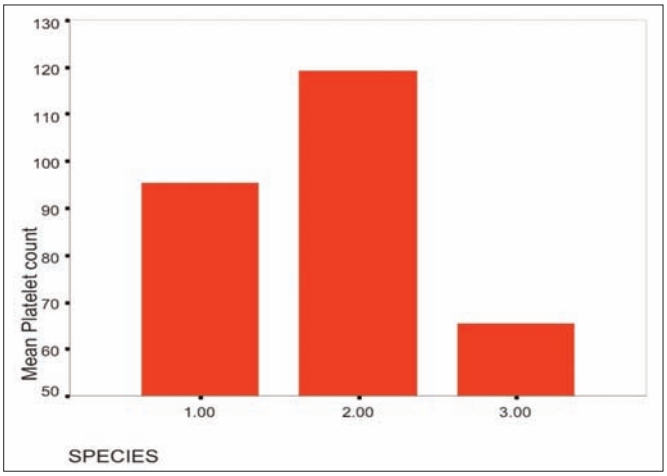

Figure 1: Mean platelet count in all types of malaria:
1. *Plasmodium falciparum*, 2. *Plasmodium vivax*, 3. Mixed *falciparum* and *vivax infection*.

DISCUSSION

Different studies in Pakistan have found a wide variation in the distribution of malaria in different regions that may be due to local factors influencing mosquito breeding, health education, malaria preventive programs, and a genetic propensity to develop malaria immunity. In this study, the frequency of malaria among the cases of fever was 11.72%, which was lower than some earlier studies that reported frequencies of 34.2% in Central Balochistan,[7] and 47% in Karachi.[2] This study was comparable to the study conducted by Khadim that showed malaria in 11.7% in the general population of Balochistan, which is the province adjoining Karachi where the present study was conducted,[3] and also to Harani that showed a malaria positivity of 12.85% in Karachi.[8]

In this study, the frequency of *P. vivax* was higher (52%) as compared to *P. falciparum* (42%). It was comparable to a study conducted by Bega, *et al.* in a tertiary care hospital in Karachi which showed *P. vivax* in 52% and *P. falciparum* in 46% of patients with acute malaria.[9] In the same study, *P. vivax* was detected in 54% and *P. falciparum* in 39% in the pediatric age group which was in contrast with an earlier study conducted at our hospital by Jalaluddin which showed a higher frequency of *P. falciparum* as compared to *P. vivax* (65% vs. 35%) in children.[10] In the adult age group, the present study revealed more cases of malaria in adults (59%) as compared with the paediatric age group (41%). It has been reported in the past that the incidence of malaria in endemic areas falls as people grow older, suggesting that advancing age contributes to immunity. But here the disease was seen in all age groups particularly in adults.

It is generally accepted that protective immunity effectively prevents the severe clinical manifestations of *Plasmodium falciparum* infections and substantially reduces parasite loads, but does not prevent infection.[11] Two forms of anti-malaria immunity has been reported; anti-parasitic i.e. the ability to control parasite densities and antitoxic immunity i.e. suppression of disease symptoms despite infection. It has been proposed that antitoxic immunity is most efficient in childhood and declines with age.[12] Also, most of the people in this study population were farmers by profession. They work in the fields from early in the morning till sunset and mostly work without using mosquito repellents and protective measures. This might be one of the factors of high malaria frequency among adults.

In this study, about 74% of the patients had haemoglobin levels of 12.0 g/dl or less and about 13.44% of the patients had haemoglobin levels of less than 6.0 g/dl (Table I). The existing literature of malaria shows anaemia rates as low as 4% and as high as 25% respectively.[1,4] The causes of anaemia in malaria are multi-factorial. It may be due to intravascular haemolysis, splenic removal of the infected cells, immune complex adsorption onto erythrocyte membranes, effects of therapeutic agents on parasitized cells and bone marrow erythroid hypoplasis.[13] Furthermore, some observers have suggested that malaria-related anaemia is more severe in the areas of intense malaria transmission and in younger children rather than older children or adults.[14] The haemoglobin changes observed in this study population may reflect a higher prevalence of underlying anaemia, poor nutritional status and non-availability of proper treatment.

Twenty-one percent (76) patients had leucopenia with a total leukocyte count of less than 4.0 x 10^3 /ul. About 9% (35) of the patients had leucocytosis with total leukocyte counts of greater than 11.0 x 10^3/ul. Among the patients with leucocytosis, 9 patients (2.4%) had total leukocyte counts of more than 20.0 x 10^3/ul. A mild to moderate leucopenia characterized by decreased neutrophils, left shift and moncytosis has been reported for malaria.[13,15] Leucopenia is thought to be due to the localization of leucocytes away from the peripheral circulation, splenic sequestration and other marginal pools rather than actual depletion or stasis.[15] Leucocytosis may suggest co-existing viral infection particularly in the presence of atypical lymphocytes common in children with concurrent viral infections.[16] Many recent studies also show leucocytosis among the malaria patients. Adedapo *et al.* reported leucocytosis in about 9.5% of the patients with malaria.[17] Leukocytosis may also have some relation with poor prognosis of disease, in relation to the value of leucocytosis in malaria. Studies have been conducted in *P. falciparum*-infected African children with similar results showing poor prognosis.[16] A co-existing viral infection should always be considered in patients presenting with acute malaria and leucocytosis. In case of neutrophilic leukocytosis, intravascular hemolysis, disseminated intravascular coagulation or additional bacterial infection must be investigated.

In this study, about 70% of the patients with malaria had thrombocytopenia with a platelet count of less than 150 x 10^3/ul (Table II). These results are comparable to many earlier studies reporting thrombocytopenia. Memon has reported thrombocytopenia in malaria to be about 70%.[2] In the present study, about 77% of *P. falciparum* and about 62% of *P. vivax* patients had thrombocytopenia. Earlier studies confirm the incidence of thrombocytopenia to be higher in *P. falciparum* malaria. In the study by Nadeem, *et al.* thrombocytopenia was observed in 83% of *P. falciparum* patients and in 70% of *P. vivax* patients.[18] In this study, 95% of the patients having mixed infection with *P. falciparum* and *P. vivax* had thrombocytopenia. Thrombocytopenia in mixed infection has also been reported in previous studies. Taha *et al.* reported the mean platelet counts to the lowest in cases of mixed infection as compared with *P. falciparum* and

P. vivax alone.[19] Platelets may play a role in the pathophysiology of severe malaria. Malaria is associated with a pro-coagulant tonus characterized by thrombocytopenia, activation of coagulation cascade and fibrinolytic system. However, bleeding and haemorrhage are uncommon, suggesting that a compensated state of blood coagulation activation occurs in malaria.[20] Maximum thrombocytopenia occurred on the fifth or sixth day of infection, and gradually returned to normal within 5-7 days after parasitemia ceased.[21] The degree of thrombocytopenia has been considered a criterion of disease severity by David, *et al.* in the United Kingdom.[22] Thrombocytopenia may result from a shortened life span of the platelets or from pooling and destruction in the spleen.[23] Platelet activation is a recent mechanism which has been suggested to account for the platelet findings in human malaria. Studies have shown that platelet activation occurs regularly in acute malaria in man, in experimental animals and in *in vitro* models. Some mechanisms of activation such as the ADP mechanism (including loss of refractoriness induced by exposure to sub-optimal ADP concentration) and sialic acid loss have been investigated and proposed.[24] Oxidative stress damage of thrombocytes has also been implicated in the etio-pathogenesis based on the finding of low levels of platelet superoxide-dismutase and glutathione peroxidase activity and high platelet lipid peroxidation levels in malaria patients, when compared to those of healthy subjects.[25] However, many details of these mechanisms, especially at the molecular level, await investigation.

The incidence of disseminated intravascular coagulation (DIC) is reported in malaria in about 4-13% of the cases and usually occurs in patients with *P. falciparum* infection and hyperparasitemia.[19] Laboratory criteria pathognomonic for DIC include evidence of procoagulant activation, thrombocytopenia, elevated prothrombin time, partial thromboplastin time or thrombin time, and decreased fibrinogen; evidence of fibrinolytic activation, elevated fibrinogen/fibrin degradation products, and elevated D-dimer; consumption of coagulation inhibitors, low levels of protein C, protein S, and antithrombin; and biochemical evidence or organ damage. Clinical evidence from endemic areas of malaria indicate that patients with severe malaria exhibit lower levels of protein C, protein S, and anti-thrombin III in comparison with patients with mild malaria. Consistent with the most common association of severe malaria cases with *Plasmodium falciparum* infection, individuals infected with *Plasmodium falciparum* exhibit lower levels of coagulation inhibitors in comparison with *Plasmodium v.vivax* infected individuals. Decreased levels of protein C have also been correlated with elevated levels of Tumor necrosis factor (TNF) in *Plasmodium falciparum* malaria.[26]

Drugs used in the treatment of *P. falciparum* malaria can causes thrombocytopenia, bone marrow suppression and hemolytic anemia, all of which can interfere indirectly with blood coagulation.[27]

CONCLUSION

The frequency of malaria in patients from Gaddap Town was 11.72%; *P. vivax* being higher (52%) as compared to *P. falciparum* (42%). Thrombocytopenia was the most common haematological finding among malaria patients. Platelet counts were lower in case of mixed *P. falciparum* and *P. vivax* infection as compared to an isolated infection of either species. Platelet counts did not drop below the critical levels of 10 X 10 3/µl in any of the patients.

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