# Alteration of Coagulation Profile in Malaria Patients and its Correlation with The Degree of Parasitemia

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## ABSTRACT

***Objective:*** To determine the alteration of coagulation profile (PT, APTT, D-dimers) in malaria patients and its correlation with the degree of Parasitemia.

***Study Design:*** Cross-sectional study.

***Place and Duration of Study:*** Pathology department of Combined Military Hospital Lahore, from Jan to July 2018.

***Methodology:*** A total of 92 patients were included in the study. The malarial parasite was identified using thick and thin smears. Hemoglobin, Red blood cell, White blood Count, Prothrombin Time, Activated Partial Thromboplastin Time, and D-dimer levels were measured using automated analyzers Sysmex KX-21 and CA-600.

***Results:*** The mean age of the patients was 23.35 ± 14.4 years with 53 (57.6%) males and 39 (42.4%) females. Plasmodium falciparum was identified as the causative species in 33 (35.9%) of the cases, 59 (64.1%) cases were caused by Plasmodium vivax. Based on parasitic load, 50 (54.3%) patients were found to have a mild degree of parasitemia whereas 26 (28.3%) had moderate and 16 (17.4%) had severe parasitemia. A significant association was found between the degree of parasitemia and coagulation parameters.

***Conclusion:*** Partial thromboplastin time, activated partial thromboplastin time and D-dimer levels were positively correlated with the degree of parasitemia specifically in plasmodium falciparum as compared to the other species. Therefore, special care should be exercised in patients having an underlying inherited bleeding disorder and a strict prevention protocol should be made.

**Keywords**: Activated partial thromboplastin time (APTT), Anemia, Hemoglobin, Malaria, Partial thromboplastin time (PT), Plasmodium falciparum, Plasmodium vivax, Platelets.

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## INTRODUCTION

Malaria istransmitted by the bite of female Anopheles mosquito.It is a leading cause of mortality and morbidity in developing countries.[1] Plasmodium species infecting the humans are further categorized into five subtypes which include *P.falciparum, P.vivax, P.ovale, P.malariae* and *P.knowlesi. P.falcipar-umcarries* the highest mortality rate.[2] It usually presents with anemia, jaundice, disseminated intravascular coagulation, acidosis (DIC) and acute renal failure.[3]

Various hematological changes have been described in patients suffering from malaria. The pathophysiology of the hematological changes in malaria patients is complex and multifactorial. Therefore, these are not fully understood and require a great deal of further research.[4] Factors that are found to affect the hematological alterations in patients affected with plasmodial infection include the prevalence of malaria, underlying hemoglobin disorder, demographic factors, and immunity against malarial parasite.

Reduced blood cell counts & mild to moderate reduction in platelet count arecommon features of malarial infection. The underlying cause of thrombocytopenia is poorly understood, however, various explanations include autoimmune destruction, sequestration in the spleen, and bone marrow aplasia with reduced platelet production. Alteration in the hemostasis plays a significant role in the progression of the disease. The severity of infections leads to accelerated activity of coagulation cascade and increased turnover of fibrinogen which ultimately leads to bleeding episodes and culminatesin DIC.[5]

The objective of this study was to assess the relationship between the degree of parasitemia and levels of PT, APTT, and D-dimers level in patients diagnosed with malaria.

## METHODOLOGY

It was a cross-sectional study, carried out in the department of Pathology in Combined Military Hospital Lahore, from Jan to July 2018. A sample size

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of 92 was calculated using online bio Mathsample size calculator (source: G.W. Snedecor& W.G. Cochran) considering the correlation coefficient of 0.29 taken from a study conducted by Ourives *et al*,[6] alpha of 0.05, Power of test 80% and confidence interval of 95%.The sampling technique used was on-probability consecutive sampling.

**Inclusion criteria:** All the cases of malaria, irrespective of age, and gender, which were newly diagnosed were included in the study.

**Exlclusion criteria:** Patients taking any medications especially antimalarial drugs having other hematological disorders, immune-compromised conditions, and acute or chronic liver conditions were excluded from the study.

This study was started after taking approval from the ethical review committee (134/2019). Informed consent was taken from the patients whose samples were collected 3ml of blood was drawn through venepuncture under aseptic conditions into EDTA containing tube. Another 2.5ml of blood was collected in a tube containing 3.8% trisodium citrate coagulant.

Thick smear for malarial parasites were prepared by placing a small drop of blood in the center 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 37oC before staining. The blood smear slides were made and stained with Leishman's stain as described by Bain and Lewis. The slides were examined by the researcher along with qualified pathologists and malarial parasites were identified. Once the presence of parasites was confirmed, a thin film was examined for recognizing the species Plasmodium falciparum, Plasmodium malaria, Plasmodium ovale, and Plasmodium vivax. The smears were examined under 40x and 100x objective oil immersion for species identification. For Malarial parasite identification on smears there were two observer for examination of smear and it required atleast 30-40 min examination according to WHO criteria of malarial species identification.

Estimations of malaria parasites based on thick and thin smear for parasite density against 200 WBCs were done and expressed as the number of parasites per microliter of blood, assuming an average WBC count of $8x10^9$/L of blood multiplied by 100. Several malaria parasites per microliter of blood = number of parasites × (8,000/number of WBCs counted).[7]

Basic hematological parameters (hemoglobin (Hb%), white blood cell count (WBC), and platelet counts were generated through an automated hematology analyzer (Sysmex KX-21). Coagulation profile including prothrombin time (PT), activated partial thromboplastin time (APTT) and D-dimer levels were measured using an automated coagulation analyzer (CA-600).

Data was entered using Statistical package for the social science version 23.0. Mean and SD were calculated for numerical variables such as age, Hb, blood cell counts including RBC, platelet, TLC. Percentage and Frequency was calculated for categorical variables like gender and type of malarial parasite. Data were divided into groups based on the type of Plasmodium parasite identified as a causative agent and degree of parasitemia.Comparison between these groups and hematological parameters was calculated using independent samples t-test and one-way ANOVA. Pearson correlation was used to signify the relationship between the degree of parasitemia and coagulation profile (PT, APTT, and D-dimer) consid-ering the *p*-value of ≤0.05 to be statistically significant.

## RESULTS

Patients were categorized into three types based on Degree of parasitemia (low, moderate, high) +1-10 Parasites per 100 thick film fields ++ 11-100 Parasites per 100 thick film fields +++ 1-10 Parasites per thick film field ++++ More than 10 Parasites per thick film field. The coagulation profile including PT with control 12 sec, APTT with control 32 sec and D-dimers with normal range <200 ng/l.zA total of 92 patients were included in this study who met the inclusion criteria. The age of the patients ranged from 3-67 years, mean age was 23.35 ± 14.4 years. Males accounted for (57.6%) of the study population and (42.4%)were females.

Plasmodium falciparum was identified as the causative species in 33 (35.9%) of the cases and 59 (64.1%) cases were caused by Plasmodium vivax. Based on parasitic load 50 (54.3%) patients were found to have a mild degree of parasitemia whereas 26 (28.3%) had moderate and 16 (17.4%) had severe parasitemia.

A comparison of the hematological parameters and parameters used for coagulation profile analysis between plasmodium falciparum and plasmodium vivax specie was done as shown in Table-I. Hemoglobin concentration and RBC count was significantly increased in patients having plasmodium vivax malaria as compared to those with plasmodium falciparum

specie ($p$=0.001). TLC however was reduced in these patients ($p$=0.001).

**Table-I: Comparison of hematological and coagulation parameters in both species.**

| | *p*.Falciparum n=30 | *p*.Vivax n=59 | *p*-value |
|---|---|---|---|
| Hb (g/dl) | 10.24 ± 0.44 | 11.33 ± 0.30 | 0.001 |
| RBC (x106 /µL) | 4.1 ± 0.09 | 4.4 ± 0.11 | 0.001 |
| Platelet (x103/µL) | 94.8 ± 4.86 | 93.6 ± 5.54 | 0.37 |
| TLC (x103/µL) | 7.07 ± 0.39 | 6.30 ± 0.11 | 0.001 |
| PT (sec) | 11.78 ± 0.99 | 12.03 ± 0.92 | 0.27 |
| APTT (sec) | 39.34 ± 4.24 | 39.59 ± 4.36 | 0.50 |
| D Dimers (ng/l) | 261.533 ± 9.25 | 262.69 ± 9.02 | 0.57 |

Analysis of variance was used to assess the effect of the degree of parasitemia on Hb, blood cell counts, and coagulation profile (Table-II). Coagulation parameters (PT, APTT, D Dimers) TLC and platelet counts were significantly affected by degree of parasitemia. An increasing trend was seen among these parameters (Table-II).

**Table-II: Alterations in hematological and coagulation parame-ters related to degree of parasitemia**

| Parameters | Mild Parasitemia (n=50) | Moderate Parasitemia (n=26) | Severe Parasitemia (n=16) | *p*-value |
|---|---|---|---|---|
| Hb (g/dl) | 10.86 ± 0.64 | 10.9 ± 0.65 | 11.13 ± 0.55 | 0.356 |
| RBC ($x10^6$ /µL) | 4.31 ± 0.18 | 4.38 ± 0.17 | 4.39 ± 0.13 | 0.178 |
| Platelet ($x10^3$ /µL) | 98.1 ± 1.07 | 88.08 ± 1.2 | 90.5 ± 6.13 | 0.001 |
| TLC ($x10^3$ /µL) | 6.65 ± 0.47 | 6.39 ± 0.27 | 6.49 ± 0.46 | 0.034 |
| PT (sec) | 11.3 ± 0.5 | 12.31 ± 0.49 | 13.5 ± 0.51 | 0.001 |
| APTT (sec) | 38.08 ± 4.1 | 40.5 ± 3.8 | 43.56 ± 2.36 | 0.001 |
| D Dimers (ng/l) | 256.2 ± 4.33 | 264.8 ± 3.3 | 278 ± 4.4 | 0.001 |

Alterations in hematological and coagulation parameters related to degree of parasitemia Post hoc analysis revealed a significant difference among various groups (Table-III).

**Table-III: Post Hoc Tukeysanalysis for intergroup comparison.**

| | Mild vs Moderate | Mild vs Severe | Moderate vs Severe |
|---|---|---|---|
| Platelet ($x10^3$/µL) | 0.001 | 0.001 | 0.01 |
| TLC ($x10^3$ /µL) | 0.03 | 0.3 | 0.7 |
| PT (Sec) | 0.001 | 0.001 | 0.001 |
| APTT (Sec) | 0.02 | 0.001 | 0.03 |
| D Dimers (ng/l) | 0.001 | 0.001 | 0.001 |

## DISCUSSION

Widespread variation in the causative agents of malaria has been well documented in Pakistani population owing to factors such as variation in harboring capacity of mosquito, health awareness, malaria prevention programs, and a genetic tendency to develop malaria immunity. Patients presenting with any febrile illness or multi-organ failure should be screened for malaria as a differential diagnosis.[8] It has been suggested that most of the malarial patients are under diagnosed despite of having increased parasitemia. It is imperative to have better diagnostic tools for malaria, particularly in the population at an increased risk of contracting the disease such as pregnant women and immune-compromised patients.[9]

In our study, the frequency of *P. viv*ax was higher (64.1%) as compared to *P. falciparum* (35.9%). Similar outcomes were reported in a study conducted by J Muhammad and colleagues in Khyber agency showing a higher prevalence of *P. vivax* in patients presenting with malaria-like symptoms.[10] Khan and coworker also report an increased incidence of *P.vivax* (97.3% ) than that of *P.falciparum* (2.6%) while no case of *P.malariae* and *P. ovale* was detected.[11]

Abnormalities in the complete blood counts are considered to be the hallmark of malarial infection. In our study, the patients presenting with Plasmodium falciparum showed a greater degree of anemia (i.e, Hb 10.14 ± 0.34 g/dl) as compared to those infected with plasmodium vivax (11.33 ± 0.30 g/dl) ($p$=0.001). However, anemia as low as 4% and as high as 25% have been reported.[12] Haroon *et al,* also reported contrasting results with anemia more prevalent in the plasmodium falciparum group.[13]

One of the commonly occurring clinical entity in the patients having malaria, for which the causative agent is plasmodium falciparum is thromobocytopenia. Many theories have been proposed regarding the underlying cause of thrombocytopenia in patients with malaria, but still there is a great need of research to be done on this topic. Thrombocytopenia was noted in our study population with a mean platelet count of 94.8 ± 4.86 $x10^3$/µL in plasmodium falciparum group and 93.6 ± 5.54 $x10^3$/µL in plasmodium vivax group ($p$=0.001). In a study conducted by Gupta *et al*, regarding platelet index and count estimation and showing their potential role to predict severity in malaria, concluded that thrombocytopenia was seen in 32 (86.4%) of *P. falciparum* and 105 (90.5%) of *P.vivax* malaria cases.[14] A systematic review conducted by Naing *et al*, concluded that thrombocytopenia is more commonly associated with plasmodium vivax infec-tions.[15]

Activation of the coagulation pathway has been associated with the plasmodium falciparum infection in patients with all age groups. Various studies have been performed but a great variation was seen in their results. This may be attributed to the relatively small sample size and variable characteristics of patients related to age, ethnicity etc, observed at the time of enrollment into the study. An overall similar observation was however made that plasmodium falciparum caused significant activation of the coagulation pathway. This activation has been directly related to the severity of the disease. Antithrombin complexes, Thrombin levels and the degradation products of fibrin were seen to be raised. Whereas protein C, protein S show a gradual decrease.

Activation of coagulation cascade plays a crucial role in the pathogenesis of malaria via occlusion of small vessels and attachment of red blood cells infected with the malarial parasite to endothelial cells.[16] Evaluation of the effect of the degree of parasitemia on hematological and coagulation parame-ters was done in this investigation. It was seen that degree of parasite load did not affect the Hb, Rbc, and WBC count. However, platelet count and the coagulation profile (PT, APTT, and D dimers) were markedly influenced by the parasitic load. In a study reported by Sirak *et al*, reported that Rbc count, Hb, and Haematocrit levels were markedly reduced in the high parasitemia group as compared to the moderate and mild groups.[17] Our study revealed a positive correlation of degree of parasitemia with thrombocy-topenia, prolongation of PT, APTT, andelevation of D-dimers. As the parasite load increases,the values of PT, APTT, and D dimers also increase showing a marked derangement in coagulation and leading to symptoms such as bleeding tendency. The coagulation profile results concluded that their alteration is not dependant on malarial species but our study shows that plasmodium falciparum and vivax both showed greater coagulation derangement.

A study conducted by Muhammad and coworkers found increased levels of D dimers, PT, and APTT.[18] Riedl *et al*, also revealed a statistically significant derangement in Plasma thrombin levels in patients with parasitic infection.[19] It was suggested that patients suffering from cerebral malaria may end up in DIC or cerebral hemorrhages. Theyrecommend that patients infected with malarial parasite should avoid the use of any drugs which directly or indirectly interfere with the hemostatic or coagulation pathways. They further suggested to exercise special care in patients having an underlying inherited bleeding disorder.

There is a dire need for further largescale studies in order to better understand that which of the effects related to the hemostasis are directly involved in the pathogenesis of malaria and which of them occur due to the disease progression secondarily. This will further enlighten the clinicians with an ability to provide a better and accurate treatment targeting a specific hemostatic mechanisms.

## CONCLUSION

Our findings show thatPT,APTT, and D-dimer levels were markedly raised in patients with plasmodium falciparum infection with severe parasitemiainstead of compared to those in mild or moderate parasitemia.

**Conflict of interest:** None.

**Author's Contribution**

SJ: Conception of the work, data accquistion and article writing, SN:, NUD: Criticle review of manuscript, MF: Data charting in SPSS and ananlysis, SR:, MB: Data acquistion.

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