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Basic coagulation parameters and platelet count among malaria patients attending at Addis Zemen Primary Hospital, Northwest Ethiopia

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Abstract

Background Malaria is an intravascular parasitic-related blood disease that causes bleeding, coagulopathy, and thrombocytopenia. However, limited data shows the effect of Plasmodium species infection on basic coagulation parameters and platelet count. Thus, this study aimed to assess basic coagulation parameters and platelet count among malaria patients.

Method A cross-sectional study was conducted among 240 study participants (120 cases and 120 controls) from June 1, 2021, to February 30, 2022. A convenient sampling technique was employed to select study participants. The blood sample was collected by a trained laboratory technologist for platelet counts, prothrombin time (PT), partial thromboplastin time (PTT), international normalization ratio (INR), blood film, and serological testing. The collected data were analyzed in SPSS version 23. Data were analyzed by the Mann-Whitney U test, Kruskal Wallis H, and Spearman's rank-order correlation tests. Descriptive findings were presented through median, tables, and chart. In all cases, a P-value < 0.05 was considered statistically significant.

Results The percentage of mild, moderate, and high malaria parasitemia levels per microliter of blood was 21.7%, 20%, and 58.3%, respectively. The overall median malaria parasitemia was 10,304 per microliter of blood. Among malaria patients, 77.5%, 61.7%, and 51.7% had prolonged PT, INR, and APTT, respectively as compared to control. Moreover, 26.7% of Plasmodium-infected participants had mild thrombocytopenia as compared to the control group ($P < 0.001$).

Conclusion The value of PT, APTT, and INR were significantly elevated, whereas the level of platelet count was inversely reduced when the malaria parasitemia level increased as compared to controls ($p < 0.001$).

Keywords Basic coagulation parameters, Malaria, Northwest Ethiopia, Parasitemia

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Introduction

Background

Malaria is one of the world's deadliest and life-threatening blood parasitic diseases. Globally, it is the fifth major cause of death [1, 2]. According to a WHO report, there are more than 249 million malaria cases recorded globally and 233 million cases recorded in African regions which account for 94% of cases globally [3].

In Ethiopia, where malaria is thought to be endemic over three-quarters of the country, more than 70 million people are at risk of contracting the disease. In 2016, there were over 3 million cases of malaria and 5,000 deaths associated with the disease. 30% of the Disability-Adjusted Life was due to malaria. Years lost, which poses a serious obstacle to social and economic advancement [4]. It mainly dysregulates hematological values and leads to anemia, thrombocytopenia, leukopenia, and coagulation abnormalities [5, 6].

Malaria is an intravascular disease that activates and consumes platelets leading to thrombocytopenia. On the surfaces of platelets or endothelial cells, pro- and antithrombotic pathways are intricately balanced during normal hemostasis. However, malaria disrupts the regular intravascular environment and determines the pathology in the tissues [7]. Different mechanisms, such as endothelial activation by pro-inflammatory cytokines, circulating microparticles, endothelial damage, binding between parasite-derived proteins on the surface, and coagulation-based receptors on the endothelium or circulating blood, may result in the activation of platelets, adhesion, and aggregation, and initiation of the coagulation cascade system consequently consumption of platelets [8].

The pathophysiology of thrombocytopenia results from the destruction of platelets by the spleen under the influence of malaria parasite antigen bound to the platelet surface, suppression of thrombopoiesis by malaria parasites that accesses the bone marrow, or both [9]. The intricate pathophysiology of malaria thrombocytopenia may involve coagulation abnormalities, splenomegaly and macrophage-mediated platelet destruction, antibody-mediated platelet destruction, oxidative stress, and platelet aggregation [10]. Immune-mediated destruction of IgG antibodies release of adenosine diphosphate by hemolyzed parasitized RBCs [11], disseminated intravascular coagulation (DIC), pooling within the reticuloendothelial system, sequestration in the microcirculation, and malaria-mediated apoptosis are some of the proposed mechanisms for the accelerated clearance or consumption of platelets during malarial infection [12].

In addition, malaria changes the blood coagulation system, activation of the coagulation cascade, and bleeding or thrombotic problems [8]. Accelerated fibrinogen turnover, antithrombin consumption, decreased factor XIII, and elevated concentrations of fibrin breakdown products are all indicative of an accelerated coagulation cascade activity. Common characteristics include prolonged prothrombin time (PT) and activated partial thromboplastin time (APTT) values, which are caused by reduced synthesis of procoagulant components and increased consumption of them [13]. Furthermore, in over 10% of instances of severe malaria, DIC develops, which is a major coagulopathy linked to low platelet survival [6, 14].

On the other hand, according to several studies, malaria causes bleeding tendencies [8, 15, 16], localized intravascular coagulation problems [17], and DIC [18]. Despite the high incidence, research on the impact of plasmodium species infection on fundamental coagulation parameters in infected patients is lacking in our nation. Therefore, this study is crucial to evaluate the basic coagulation parameters and platelet count among plasmodium-infected study participants compared with healthy control.

Materials and methods

Study area, period, and study design

A cross-sectional study was conducted from June 1, 2021, to February 30, 2022, at Addis Zemen Primary Hospital. The Hospital serves not only for Libo Kemkem district but also nearby districts. Study participants who were pregnant, on antiretroviral therapy (ART), and anticoagulant therapy, history of liver disease, hypertension, cardiac disease, renal disease, hepatitis, and diabetes mellitus were excluded from the study. Only microscopically confirmed adult malaria patients for the case group were included.

For the control group, apparently healthy adults who visited at voluntary counseling and testing (VCT) clinic of Addis Zemen Primary Hospital during the study period were included in the study.

Sample size determination

According to rules of thumb that have been recommended by van Voorhis and Morgan, 30 participants per group are required to detect real differences, which leads to about 80% power [19]. To increase the accuracy and reliability of the study, the study participants recommended by the rule of thumb has to be increased by four-fold. Thus, a total of 240 study participants with sex and age-matched (120 malaria cases and 120 controls) were enrolled in the study. A convenience sampling technique was employed to select study participants.

Data collection and laboratory methods

Questioner survey

Data were collected by pretested and semi-structured questionnaires using the interview technique. Socio-demographic characteristics, patient history,

clinical signs, and symptoms of patients were collected by a trained data collector and supervised by the principal investigator. A trained physician and nurse assessed clinical information and physical diagnosis of malaria-negative adults who attended at VCT clinic. Before data collection, data collectors selected individuals who fulfilled inclusion criteria and obtained consent from each study participant.

Blood film

Both thick and thin blood smears were prepared on a frosted microscopic slide for each acute febrile patient from capillary blood by finger prick using a sterile lancet. The blood film was allowed to air-dry at room temperature and the absolute methanol was used to fix the thin blood film. Then, the blood film was stained with 10% Giemsa solution for 10 min and examined by an experienced medical parasitologist.

The presence of Plasmodium species was ruled out if no parasites were observed after examination of at least 100 microscopic fields with a hundred (100X) objective. Both thick (parasite detection and quantification) and thin blood (malaria species identification) smear was used. Malaria parasitemia was estimated in thick film by counting the number of asexual parasites (both trophozoite and schizont stage of malaria) along with 200 white blood cells (WBC). Malaria parasitemia level expressed as the number of asexual parasites per μL of blood was calculated by dividing the number of asexual parasites by the number of WBCs counted and then multiplying by WBC per μL of blood.

$$\text{Malaria parasitemia level}/\mu\text{L} = \frac{\text{Number of parasites counted} \times \text{WBC count}/\mu\text{L}}{\text{Number of WBCs counted}}$$

Two Medical parasitology professionals read all the slides independently and parasite densities were calculated by averaging the two counts. Blood smears with discordant results (differences between the two parasitology professionals in species diagnosis were re-examined by a third independent parasitologist, and parasite density was calculated by averaging the two closest counts [20].

The level of malaria parasitemia was classified as mild less than 1000 parasites/μL blood, moderate between 1000 and 4999 parasites/μL blood, and severe parasitemia greater than 5,000 parasites/μL blood [21].

Blood collection and laboratory analysis

Blood sample collection

Both capillary and venous blood samples were collected from each selected study participant simultaneously. Capillary blood was collected from each participant by puncture with a sterile blood lancet from the left hand of the middle figure. The blood was collected with a sterile pasture pipette after wiping the first flow of blood. Both thin and thick films were prepared for the identification of plasmodium species.

Furthermore, five milliliters (ml) of venous blood were collected by following standard operating procedures by a qualified laboratory technologist together with the principal investigator. The collected blood sample was dispensed into two test tubes. The first 2.7 mL was transferred to a 3.2% sodium citrate anticoagulated test tube for PT, APTT, and INR test analysis. The remaining amount of blood was transferred to Ethylene Diamine Tetra-Acetate (EDTA) test tubes for platelet count, Hepatitis B Surface Antigen, and Hepatitis C virus testing. Blood with citrated anticoagulant was centrifuged at 1500 revolutions per minute for 15 min for the determination of PT and APTT tests. The INR value of each participant was calculated based on the value of the patient PT result, the PT of the control, and the manufacturer's international Sensitivity Index (ISI) according to the following formula.

$$\text{INR} = \left(\frac{\text{Patient's PT}}{\text{Control PT}}\right)^{\text{ISI}}$$

Laboratory analysis for blood sample

The platelet count was done by Sysmex KX-21 automated hematology analyzer (Sysmex Corporation Kobe, Japan) within 1 h of blood sample collection. After the result was obtained, we classified the platelet count into normal (>150,000 cells/mm3), mild thrombocytopenia (75,000–144,999 cells/mm^3), moderate thrombocytopenia (50,000–74,999 cells/mm^3), and severe thrombocytopenia (25,000–49,999 cells/mm^3).

Similarly, a basic coagulation profiles test (PT, APTT, and INR) was performed from platelet-poor plasma by using HumaClotDue^{plus} coagulation analyzer (Wiesbaden, Germany) at Gondar University Hospital laboratory. Laboratory tests were performed by a trained senior laboratory technologist together with a principal investigator at Addis Zemen Primary Hospital and Gondar University Hospital laboratory. We interpreted our laboratory results based on the reference interval of PT (10–14 s), APTT (24–36 s), and INR (0.8–1.2) results.

Serological tests like Hepatitis B surface antigen test (Core tests, China), Hepatitis C virus test (*Cortez Diagnostic, Inc*), and Urine HCG test (female) (Guangzhou Wondfo Biotech Co Ltd, Guangzhou, China) were done for each selected participant. Positive results were excluded from being sampled.

Quality control

To maintain the quality of data, training was given to the data collectors on how to collect, process samples, and record results. Principal investigators were continuously supervised and worked with data collectors. To ensure the accuracy of the parasite counts, two qualified laboratory technologists read all the slides independently, and parasite densities were calculated by averaging the two counts. Moreover, the quality of reagents such as Giemsa stains was checked using the known positive and negative samples every day before starting daily work.

The quality of test results was maintained strictly by following laboratory standard operating procedures. For platelet count, a regular background check was done to minimize background error. Normal and abnormal controls were run for each laboratory test following the manufacturer's recommendations. The investigator followed and frequently checked every process to ensure the completeness and consistency of the collected data.

Data management and analysis

After checking the completeness, data were coded and entered into EpiData Manager (v4.4.2.1) statistical software and then exported to SPSS version 23 software for analysis. The Kolmogorov-Smirnov and Shapiro-Wilk normality tests were used to check the normality distribution of continuous variables.

Nonparametric tests, the Mann-Whitney U test was used to compare the median difference in basic coagulation profiles and platelet count between cases and controls. Kruskal Wallis H and Dunn-Bonferroni pairwise comparison were used for the comparison of coagulation profiles and platelet count between different parasitemia levels. Spearman's rank correlation analysis was used to assess the correlation of a number of asexual developmental stages of parasites per μL of blood with basic coagulation profiles and platelet count. The results were presented as the median and interquartile range (IQR) for each group. Frequency, tables, and chart was used to present the summarized data. In all statistical analysis, a p-value < 0.05 was considered a statistically significant association.

Results

General characteristics of study participants

A total of 240 adult individuals were included in the present study. The age of the study participants ranged from 18 to 52 years old with the median age of the study participant being 28 years old (IQR: 10 years old). The proportion of male study participants in the case group and control group was 80 (66.7%) and 77 (65.4%), respectively. A majority of study participants were orthodox in religion 212 (88.3%), living in rural areas 132 (55%), and farmers in their occupation 63 (26.3%) (Table 1).

Detection of *Plasmodium species* and parasitemia level among case group

Among all plasmodium-infected patients 63 (52.5%), 43 (35.8%), and 14 (11.7%) of the participants were infected by *Plasmodium falciparum*, *Plasmodium vivax*, and mixed infection, respectively. The percentage of mild, moderate, and high malaria parasitemia levels per microliter of blood was 21.7%, 20%, and 58.3%, respectively.

For individuals with *Plasmodium* species infection, the overall median (interquartile range) parasitemia of asexual stages of the parasite was 10,304 (19166) per microliter of blood. The median parasitemia of *Plasmodium* species infection among male and female study participants was 10,304 and 10,584.5 per microliter of blood, respectively. It didn't show a statistical difference between male and female participants ($p=0.896$). The highest and lowest parasite counts per microliter of blood were 112,000 and 80, respectively. The highest parasitemia levels of *Plasmodium falciparum*, *Plasmodium vivax*, and mixed infection were 60.3%, 58.2%, and 50%, respectively (Fig. 1).

Basic coagulation profiles and platelet count of the study participants

Based on our data, the percentages of prolonged PT, INR, APTT, and thrombocytopenia of malaria patients were higher than apparently healthy individuals. Among malaria patients, 93 (77.5%), 74 (61.7%), and 62 (51.7%) had prolonged PT, INR, and APTT, respectively. On the other hand, 26.7% (95% CI: 19.2-35%) of *Plasmodium*-infected adults had mild thrombocytopenia. From thrombocytopenia patients, the percentage of *Plasmodium falciparum*, *Plasmodium vivax*, and mixed infection was 53.1%, 40.6%, and 6.3%, respectively. Based on the severity of thrombocytopenia, only a mild type of thrombocytopenia was present in the study participants. Among healthy controls 6 (5.0%), 4 (3.3%) and 7 (5.8%) had prolonged PT, INR, and APTT, respectively; whereas, only 2 (1.7%) had thrombocytopenia (Table 2).

Comparison of basic coagulation profiles and platelet count among groups

The minimum versus the maximum value of PT, APTT, INR, and platelet counts among the case group were 12.6 vs. 22.8 s, 28.3 vs.43.8 s, 1.04 vs.2.1, and 98 vs. 402 cells per microliter blood, respectively. Whereas, the minimum versus maximum values of PT, APTT, INR, and platelet counts among the control group were 10 vs. 17.8 s, 24.9 vs. 38.9 s, 0.94 vs.1.58, and 133 vs. 398 cells per microliter of blood, respectively. The median values of PT, INR, and APTT in the case group were greater than the median value of PT, INR, and APTT in the control group. However, the result of platelet count in the

Table 1 Socio-demographic characteristics of study participants at Addis Zemen Primary Hospital general OPD and VCT clinic (n = 240) from June 1, 2021 to February 30, 2022

Socio-demographic characteristics	Malaria patients n = 120		Healthy control n = 120		Total n = 240	
	Frequency	%	Frequency	%	Frequency	%
Sex						
Male	80	66.7	77	64.2	157	65.4
Female	40	33.3	43	35.8	83	34.6
Age						
18–24	40	33.3	26	21.7	66	27.5
25–34	54	45.0	63	52.5	117	48.8
35–44	17	14.2	24	20.0	41	17
45–54	9	7.5	7	5.8	16	6.7
Residence						
Urban	51	42.5	57	47.5	108	45.0
Rural	69	57.5	63	52.5	132	55.0
Religion						
Orthodox	107	89.2	105	87.5	212	88.3
Muslim	13	10.8	15	12.5	28	11.7
Occupation						
Student	25	20.8	31	25.8	56	23.3
Gov't employee	5	4.2	7	5.8	12	5.0
Daily laborer	23	19.2	15	12.5	38	15.8
House wife	22	18.3	18	15.0	42	16.7
Farmer	31	25.8	32	26.7	63	26.3
Merchant	14	11.7	17	14.2	31	12.9
Educational status						
Illiterate	44	36.6	41	34.2	85	35.4
Primary school	45	37.5	42	35.0	87	36.3
Secondary school	26	21.7	24	20.0	50	20.8
Diploma	5	4.2	14	10.8	18	7.5
Marital status						
Single	55	45.9	64	53.3	119	49.6
Married	46	38.3	25	20.8	71	29.6
Divorced	12	10.0	26	21.7	38	15.8
Widowed	7	5.8	5	4.2	12	5.0

case group was lower than the result of the control group (p-value < 0.001) (Table 3).

The median [IQR] value of PT, INR, and APTT were prolonged and platelet count was decreased in *Plasmodium*-infected adults. A Mann-Whitney U test revealed a significant difference in the median of PT, INR, APTT, and platelet count of *Plasmodium*-infected adults and healthy control participants ($p < 0.001$) (Table 3).

Comparison of basic coagulation profiles and platelet count based on the infection of *Plasmodium* species

In the Kruskal-Wallis H comparison, the median [IQR] of PT, INR, APTT, and platelet count didn't show statistically significant differences among *Plasmodium falciparum*, *Plasmodium vivax*, and mixed infection groups ($p > 0.05$ in each) (Table 4).

Comparison of basic coagulation profiles and platelet count based on the malaria parasitemia levels

Based on the Kruskal-Wallis H comparison, the median [IQR] of PT, INR, APTT, and platelet count showed statistically significant differences among low, moderate, and high malaria parasitemia group ($p < 0.001$ in each). The high malaria parasitemia group recorded the highest median score of PT, INR, and APTT than the other groups. Whereas the high malaria parasitemia group recorded the lowest median value of platelet count than the other groups.

In multiple pairwise comparisons analysis using the Dunn-Bonferroni pairwise comparison test, the median [IQR] values of PT, INR, and APTT of the high malaria parasitemia group were significantly higher than other parasitemia groups ($p < 0.001$). Similarly, the median [IQR] value of platelet count was significantly lower in the high parasitemia group compared to the low,

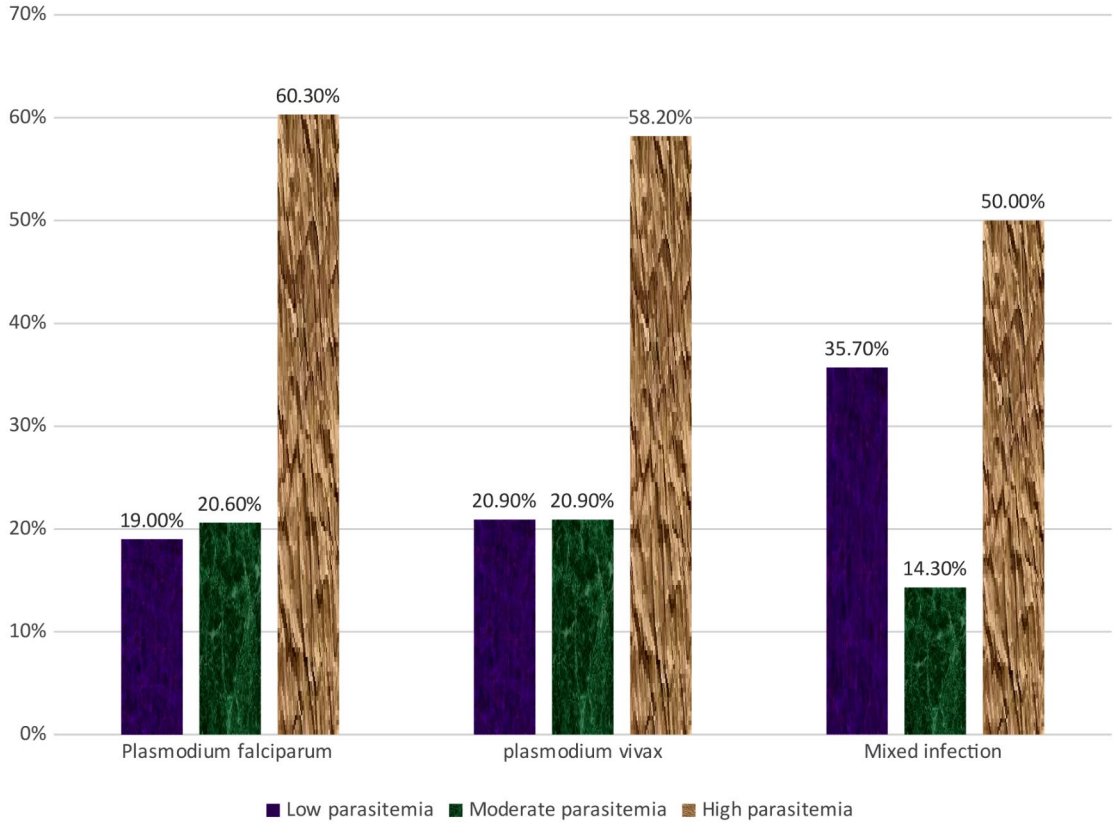

Fig. 1 The malaria parasitemia level at Addis Zemen primary hospital

Table 2 Basic coagulation profiles and platelet count of study participants at Addis Zemen Primary Hospital general OPD and VCT clinic from June 1, 2021, to February 30, 2022

Variables		Malaria patients (N = 120)				Healthy controls (N = 120)	Reference interval
		Plasmodium falciparum frequency (%)	Plasmodium vivax frequency (%)	Mixed frequency (%)	Overall frequency (%)	Frequency (%)	
PT (in Sec)	Shortened	0	0	0	0	0	10–14 s
	Normal	12 (19)	11 (25.6)	4 (28.6)	27 (22.5)	114 (95.0)	
	Prolonged	51 (81)	32 (74.4)	10 (71.4)	93 (77.5)	6 (5.0)	
INR	Shortened	0	0	0	0	0	0.8–1.2
	Normal	23 (36.5)	16 (37.2)	7 (50)	46 (38.3)	116 (96.7)	
	Prolonged	40 (63.5)	27 (62.8)	7 (50)	74 (61.7)	4 (3.3)	
APTT (in Sec)	Shortened	0	0	0	0	0	24–36 s
	Normal	30 (47.6)	20 (46.5)	8 (57.1)	58 (48.3)	113 (94.2)	
	Prolonged	33 (52.4)	23 (53.5)	6 (42.9)	62 (51.7)	7 (5.8)	
Platelet count ($x10^3/\mu L$)	Mild thrombocytopenia	17(27)	13 (30.2)	2 (14.3)	32 (26.7)	2 (1.7)	75,000-144,999/μL
	Normal	46 (73)	30 (69.8)	12 (85.7)	88 (73.3)	118 (98.3)	$150–450 \times 10^3$ /μL

PT: Prothrombin Time; INR: International Normalization Ratio; APTT: Activated Partial Thromboplastin Time; IQR: interquartile range, Sec: second

Table 3 Comparison of basic coagulation profiles among malaria patients and healthy control groups at Addis Zemen Primary Hospital general OPD and VCT clinic (n = 240) from June 1, 2021, to February 30, 2022

Variables	Healthy controls (n = 120)	Malaria patients (n = 120)	*p*-value
	Median [IQR]	Median [IQR]	
PT (in Sec)	13.35 [1.7]	15.6 [2.9]	< 0.001
INR	1.135 [0.09]	1.40 [0.42]	< 0.001
APTT (in Sec)	31.3 [3.28]	36.1 [4.05]	< 0.001
Platelet count (x10^3/μL)	267 [89]	197.5 [114]	< 0.001

PT: Prothrombin Time; INR: International Normalization Ratio; APTT: Activated Partial; Thromboplastin Time; IQR: interquartile range

Table 4 Comparison of basic coagulation profiles and platelet count based on infection of Plasmodium species at Addis Zemen Primary Hospital (n = 120) from June 1, 2021, to February 30, 2022

Variables	infection of Plasmodium species			*p*-value
	Plasmodium falciparum (n = 63)	*Plasmodium vivax* (n = 43)	Mixed infection (n = 14)	
	Median [IQR]	Median [IQR]	Median [IQR]	
PT (in Sec)	15.6 [3.0]	15.8 [2.9]	15.95 [3.32]	0.902
INR	1.42 [0.41]	1.38 [0.41]	1.30 [0.45]	0.930
APTT (in Sec)	36.1 [3.9]	36.1 [3.3]	34.8 [5.15]	0.964
Platelet count (x10^3/μL)	195 [134]	200 [113]	205.5 [78]	0.909

PT: Prothrombin Time; INR: International Normalization Ratio; APTT: Activated Partial Thromboplastin Time; IQR: interquartile range

Table 5 Comparison of basic coagulation profiles and platelet count based on malaria level of parasitemia at Addis Zemen primary hospital (n = 120) from June 1, 2021, to February 30, 2022

Variables	Malaria parasitemia level			*p*-value
	Low (n = 55)	Moderate (n = 42)	High (n = 23)	
	Median [IQR]	Median [IQR]	Median [IQR]	
PT (in Sec)	13.1 [1.1]	14.3 [0.8]	16.9 [2]	< 0.001
INR	1.1 [0.11]	1.2 [0.05]	1.6 [0.32]	< 0.001
APTT (in Sec)	32.8 [0.9]	34.5 [1.1]	37.4 [2.3]	< 0.001
Platelet count (x10^3/μL)	298 [69]	230[75]	165 [60]	< 0.001

Table 7 Correlation of the number of asexual stage parasites with basic coagulation profiles and platelet count among Plasmodium-infected adults attending Addis Zemen Primary Hospital

Variables	Correlation coefficient (r)	Significance level (p-value)
PT	0.928	< 0.001
INR	0.910	< 0.001
APTT	0.914	< 0.001
Platelet count	-0.716	< 0.001

PT: Prothrombin Time, APTT: Activated Partial Thromboplastin Time, INR: International normalization ratio, r = Spearman's rank-order correlation *(rho)*

and moderate malaria parasitemia group ($p < 0.05$). As the severity of malaria infection increased the levels of thrombocytopenia were proportionally reduced (Table 5).

However, the median [IQR] value of platelet count, INR, and APTT didn't show significant differences between low malaria parasitemia and moderate malaria parasitemia groups based on multiple pairwise comparisons ($p > 0.05$) (Table 6).

The correlation of the number of asexual stages of the malaria parasite with basic coagulation profiles and platelet counts

In malaria patients, Spearman's rank-order correlation analysis showed that the number of asexual developmental stage of parasites had a strongly positive correlation with PT, INR, and APTT values (Spearman's rho correlation coefficient $r = 0.928$, 0.910, and 0.914, respectively; ($p < 0.001$)). However, the platelet count of malaria patients was negatively correlated with an asexual stage of parasitemia level (Spearman's rho correlation coefficient $r = -0.716$, $p < 0.001$) (Table 7).

Discussion

Malaria is a life-threatening disease that remains the major cause of mortality throughout the World. It mainly dysregulates the normal blood hemostasis system leading to the consumption of platelets and coagulation factors results bleeding or thrombotic complications [22, 23]. The present study aimed to investigate the effect

Table 6 Multiple pairwise comparisons of basic coagulation profiles and platelet count based on Plasmodium parasitemia at Addis Zemen Primary Hospital general OPD (n = 120) from June 1, 2021 to February 30, 2022

Variables	L Vs M	LVs H	M Vs L	M Vs H	H Vs L	H Vs M
PT (in Sec)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
INR	0.406	< 0.001	0.406	< 0.001	< 0.001	< 0.001
APTT (in Sec)	0.35	< 0.001	0.35	< 0.001	< 0.001	< 0.001
Platelet count (x10^3/μL)	0.937	< 0.001	0.93	< 0.001	< 0.001	< 0.001

PT: Prothrombin Time; INR: International Normalization Ratio; APTT: Activated Partial Thromboplastin Time; L: low malaria parasitemia; M: moderate malaria parasitemia; H: high malaria parasitemia

of malaria on the value of PT, APTT, INR, and platelet count at Addis Zemen Primary Hospital, Northwest Ethiopia.

In this study, the value of the coagulation profile of PT (77.5%), APTT (51.7%), and INR (61.7%) in the case group was prolonged ($p<0.001$) as compared to the value of control results. This finding was similar to studies reported in Sudan [24], West Bengal, India [25], and State Karnataka, India [26], Kasala State, Sudan [7], Sudan Sudan-Sinnar Hospital [27], Khartoum state, Sudan [24], Dembia Primary Hospital and Makisegnit Health Center, Ethiopia [28], West Bengal, India [15], and India [29]. Moreover, as the level of malaria parasitemia increased; the median value of PT, INR, and APTT values were increased. This finding was supported by the study conducted in Bangalore, India [30]. It was found that the severity of malaria parasitemia increased the value of PT, APTT, and INR also increased [29]. However, This result was contradicted in the study reported in Ashanti Region, Ghana [31]. The possible difference might be due to eligibility criteria. Our study was conducted with symptomatic malaria patients as compared to the Ghana study which was asymptomatic malaria participants. During symptomatic malaria, there may be active malaria replication which increases malaria parasitemia levels that may have the potential to prolong the value of PT, APTT, and INR results as compared to asymptomatic malaria cases.

Furthermore, this study revealed that 26.7% of thrombocytopenia was found in the case groups ($P<0.001$) as compared to the control group (1.7%). However, the level of thrombocytopenia was lower as compared to Bangalore, India reported (58%) [13], India (81%) [32], and Karnataka, India (89.3%) [33]. The possible difference might be due to the use of laboratory method variations for the determination of platelet count. In this study, we used a hematology analyzer for the platelet count and capillary blood for parasite detection and quantifications. Whereas the India study reported platelet count with manual technique (platelet estimation) and malaria count was performed based on buffy coat preparation (Karnataka, India study).

Moreover, as the level of malaria parasitemia level increased, the levels of platelet counts were proportionally reduced. The result was agreed to in a study reported in Bangalore, India [29], India [34], Maharashtra state, India [35], Kankanady Mangalore, India [30], Ahmedabad, India [25], and Thailand [36]. This might be due to different mechanisms. The first mechanism might be due to antiphospholipid-mediated destruction of platelets. These antibodies are found in the serums of malaria patients and bound to platelet membrane then removed by the hyperplastic reticuloendothelial system, particularly the spleen leading to thrombocytopenia. The second mechanism might be due to the destruction of platelets by IgG antibodies' release of adenosine diphosphate by hemolyzed parasitized red blood cells [37, 38]. The third mechanism may be due to the sequestration of the injured platelets in the spleen followed by phagocytosis by splenic macrophage [39]. The fourth mechanism might be due to the damage of endothelium by malaria, which initiates platelet activation, aggregation, coagulation cascade reactions, and increased consumption of platelets during the reaction [6, 8].

Spearman's rank-order correlation test showed a significant and positive correlation between the number of asexual developmental stages of the parasite per microliter of blood and PT, INR, and APTT values of malaria patients with the correlation coefficient of 0.928, 0.910, and 0.914, respectively ($p<0.001$). This finding was agreed with a study reported in Kasala State [7]. This indicates that the parasite load increased the duration time of PT, APTT, and INR for clotting also prolonged or elevated.

In addition, there is a positive correlation between the number of asexual developmental stages of the parasites and prolongation of PT, APTT, and INR values; whereas negative correlation with platelet count ($p<0.001$). The result was concordant with the study conducted in South Ethiopia [40], Harar, Ethiopia [41], Bangalore, Karnataka [13], and Pakistan [42]. The main limitation of this study was we could not incorporate platelet function assays, or individual factor assays, and could not assess the participant's genetic abnormality other than malaria.

Conclusion

In the present study, the values of PT, APTT, and INR were prolonged as compared to the control group. The levels of PT, APTT, and INR were proportionally increased when the malaria parasitemia level increased whereas the platelet count was proportionally decreased.

The number of asexual developmental stages of the parasite per microliter of blood had positively correlated with the values of PT, INR, and APTT. However, a negative correlation was found for the platelet count. In conclusion, *Plasmodium* species infection could be one of the main factors in the development of coagulation test abnormality (PT, APTT, and INR) and thrombocytopenia. Therefore, we recommend health professionals working in Addis Zemen Primary Hospital for routine check-ups of basic coagulation profiles (PT, APTT, and INR) and platelet count to reduce further malaria-related coagulation and platelet count abnormalities.

Abbreviations

APTT	Activated Partial Thromboplastin Time
ART	Antiretroviral Therapy
DIC	Disseminated Intravascular Coagulation
EDTA	Ethylene Diamine Tetra-Acetate

INR	International Normalization Ratio
PT	Prothrombin Time
VCT	Voluntary Counseling Test
WBC	White Blood Cells
WHO	World Health Organization

Supplementary Information

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Supplementary Material 1

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Author contributions
Tahir Eyayu and Tegenaw Tiruneh was participated in data collection, investigation; methodology; project administration; resources; software; supervision; validation; visualization; writing original draft; and writing-review & editing of the paper. Aynework Abebaw: data curations; Biruk Legese: formal analysis; Teklehaimanot Kiros: funding acquisitions; Andargachew Almaw and Mesilo Sema: supervision; Shiwaneh Damte and Desalegn Andargie: project administration; Dejen Getaneh Feleke and Bernabas Andargie: resources; Ayenew Birhan: software; Tadila Dires and ESC had substantial contribution for supervision. All authors read and approved the final manuscript.

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Data availability
Data is provided within the manuscript.

Declarations

Ethics approval and consent to participate
The study was ethically approved by the Ethical Review Committee of the College of Health Sciences, Debre Tabor University (ethical review reference number DTU/RP/924/13). Moreover, a letter of support was secured from the Woreda health office and research coordinating office of the College of Health Sciences. Before starting the actual data collection, a permission letter was obtained from the hospital manager. After explaining the purpose, the benefits, and the possible risks of the study informed written consent was taken from each study participant. All the information obtained from the study participants and laboratory results were kept confidential. The study participants who tested positive for different infections and had coagulation abnormalities were linked to the physician in Addis Zemen Primary Hospital for treatment and better patient management purposes.

Consent to publish
Not applicable.

Competing interests
The authors declare no competing interests.

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