Wydawnictwo UR 2022
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2022.4.8

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2022; 20 (4): 430–434

ORIGINAL PAPER

The role of hematological parameters in differentiating *Plasmodium falciparum* and others – a study from Somalia

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ABSTRACT

Introduction and aim. Accurate identification of Plasmodium species is important because of the differences in their treatment. We aimed to investigate the role of hematological and biochemical parameters in the differentiation of *Plasmodium falciparum* and other plasmodium species.

Material and methods. This is a retrospective study. Patients admitted to the emergency department with signs and symptoms of malaria were included into the study. Patients with malaria were grouped as *P. falciparum* and others. Hematological parameters of two groups were compared by univariate and multivariate analysis. Statistical analysis was performed using the Jamovi.

Results. A total of 107 patients were included in the study. According to univariant and multivariant analysis there was no difference in between two groups in the terms of blood urea nitrogen, aspartate aminotransferase, total bilirubin, hemoglobin, hematocrit, white blood cell count, platelet count, and mean platelet volume (in univariate analysis p values were 0.029, 0.011, 0.019, 0.171, 0.870, 0.307, 0.042, and 0.276, respectively and in multivariate analysis p values for blood urea nitrogen, aspartate aminotransferase, total bilirubin, hemoglobin, and platelet count were 0.100, 0.535, 0.328, and 0.213, respectively).

Conclusion. The investigated hematological and biochemical parameters were found to be not valuable in predicting type of malaria. On the other hand, we recommend confirming the results of our study with larger samples and multicenter studies.

Keywords. malaria, Plasmodium falciparum, Plasmodium vivax, platelets

Introduction

Malaria is a disease that has been known since ancient times and is seen in 300-500 million people per year in developing countries in tropical regions. Among the infectious diseases, death due to malaria ranks 6th to 8th.[1] It is estimated that in Africa, there are more than 12,000,000 malaria cases, and 155,000 –310,000 malaria-related deaths per year attributable to epidemics if control options are not implemented or well-timed, that is equal to about 4% of estimated annual malaria cases worldwide and 12–25% of estimated annual worldwide malaria-related deaths, including up to 50% of the estimated malaria mortality (annual worldwide) in adults.[2]

The traditional diagnosis of malaria is made by examining thin smear and thick drop preparations and this is accepted as the gold standard. However, several disadvantages of this method are known. Peripheral smear examination fails during periods of low parasitemia. In order to diagnose with the traditional method, it is necessary to examine a large number of microscopic fields, which is very laborious, causes loss of time and requires experienced personnel.[3] The type of parasite, the negligence

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Received: 23.08.2022 / Revised: 9.10.2022 / Accepted: 25.10.2022 / Published: 30.12.2022

Özdemir S, Algın A, Altunok İ, Arslan E. *The role of hematological parameters in differentiating Plasmodium falciparum and others – a study from Somalia.* Eur J Clin Exp Med. 2022;20(4):430–434. doi: 10.15584/ejcem.2022.4.8.

of the microscopy, the mistakes made during the preparation and staining of blood smears sometimes cause the inadequacy of traditional diagnostic methods. For this reason, many research laboratories are trying to develop alternative methods for the diagnosis of malaria.[4]

Accurate identification of Plasmodium species is important because of the differences in their treatment. *Plasmodium falciparum* infections can be fatal in a very short time, *Plasmodium vivax* and *Plasmodium ovale* hypnozoites can remain latent in liver cells and cause relapses. The appearance of gametocytes in untreated individuals indicates active infection, and in treated individuals' persistent infection. It should be noted that persistent infection can also be seen after successful treatment.[5] Hemogram analysis is an easily accessible and inexpensive examination. We speculated that the hematological parameters may contribute to the differentiation of plasmodium species or to support the diagnosis.[6]

Aim

In this study, our aim is to investigate the role of hematological and biochemical parameters in the differentiation of *P. falciparum* and other plasmodium species.

Material and methods

Ethical approval

Approval for the study was obtained from the ethics committee of Mogadishu Somalia-Turkish Training and Research Hospital with 11 November 2021 date and 450 number. Informed consent was not obtained because the study did not include the personal information of the patients, within the knowledge of the ethics committee.

Study design

This analytical study was conducted retrospectively in a tertiary hospital emergency room with a total of 300 beds, 50 of which were intensive care units, in a sub-Saharan city Mogadishu located in the coastal Banaadir region on the Indian Ocean. During the study period, there were 350 emergency applications per day from the center where the study was conducted.

Study population

Patients who applied to the emergency department with malaria symptoms and signs between January 1, 2021, and January 1, 2022, were included in the study. Patients with haemoglobinopathy or hematological malignancy and patient with missing data were excluded from study. Patients' data were obtained from written documents and hospital computer-based laboratory system. Rapid Diagnostic Kit-Rapid Diagnostic Test (RDT) (SD Bioline Malaria Ag Pf/Pan™ RDT) (Batch No. 60952) for malaria was used for the diagnostic test of the patients. RDT is using to diagnose malaria by detecting evidence of malaria parasites (antigens) in human blood. These tests require a drop of peripheral blood. Visual readings are classically available in 20 minutes. Test results are reported as *P. falciparum* and other. The patients were divided into two groups according to these results.

Data collection

Demographics, signs, laboratory parameters and clinics of the patients were documented. Signs were recorded as splenomegaly and hepatomegaly, icterus, vomiting, diarrhea, cough, headache, and pallor. The laboratory parameters were recorded as total bilirubin, indirect bilirubin, direct bilirubin, creatinine, hemoglobin, platelet count, mean platelet volume, blood urea nitrogen, aspartate transaminase, alanine transaminase, hematocrit, and white blood cell count. The clinics of the patients were recorded as spontaneous bleeding, pulmonary edema, cerebral malaria, shock, severe acidosis, severe malarial anemia, clinical evidence of jaundice renal failure, vital organ dysfunction, and minor symptoms.

Statistical analysis

Jamovi (Version 1.6.21.0; The Jamovi Project, 2020; R Core Team, 2019) was used for statistical analysis. The similarity of the data to the normal distribution was evaluated with the Shapiro Wilk test. Categorical data were presented by number and percentage, and continuous data with median and 25th and 75th percentiles. For comparison between *P. falciparum* and other groups, chi-square test was used in categorical data and Mann Whitney U test was used in continuous data. Likelihood ratios (LRs) were calculated using sensitivity and specificity values in the evaluation of relationship between plasmodium species and laboratory parameters. Values below 0.05 were used for the significant p value.

Results

A total of 107 patients were included in the study. Seventy-five (70.1%) of the patients were male. The median age was 31 (25th and 75th percentiles: 25–50). The three most common symptoms were fever (42.1%), headache (30.8%), and vomiting (20.6%). Baseline characteristics of the enrolled patients and their distribution according to the type of malaria are shown in Table 1.

The comparisons of hematological parameters of the *P. falciparum* and other plasmodium type groups are shown in table1. In univariant analysis significant differences were identified between hematological parameters of the *P. falciparum* and other plasmodium type groups as; headache (8 (18.6%) versus 25 (39.1%), p=0.025), blood urea nitrogen (15.5 (range: 9–35) versus 10 (range: 7–22) mg/dL, p=0.029), aspartate aminotransferase (43.5 (range: 30.8–65.27) versus 28 (range: 21–48) U/L, p=0.011), total bilirubin (1.43 (range: 0.61–3.84) versus 0.56 (range: 0.33–1.28) mg/L,

Table 1. Baseline characteristics of the enrolled patients and their distribution according to the type of malaria

	Total	*Plasmodium falciparum*	*Other Plasmodium*
	n=107	n=43 (40.1%)	n=64 (59.9%)
Age, years	31 (25–50)	31.0 (25.5–51.5)	31.5 (24–48)
Gender			
Male	75 (70.1%)	30 (69.8%)	45 (70.3%)
Female	32 (29.9%)	13 (30.2%)	19 (29.7%)
Signs and symptoms			
Fever	45 (42.1%)	15 (34.9%)	30 (46.9%)
Headache	33 (30.8%)	8 (18.6%)	25 (39.1%)
Vomiting	22 (20.6%)	9 (20.9%)	13 (20.3%)
Cough	15 (14.2%)	7 (16.7%)	8 (12.5%)
Diarrhea	36 (34.0%)	15 (35.7%)	21 (32.8%)
Pallor	13 (12.1%)	6 (14.0%)	7 (10.9%)
Icterus	19 (17.8%)	7 (16.3%)	12 (18.8%)
Hepatomegaly	15 (14.0%)	9 (20.9%)	6 (9.4%)
Splenomegaly	6 (5.6%)	3 (7.0%)	3 (4.7%)
Laboratory findings			
Aspartate aminotransferase (U/L)	32 (22–57)	43.5 (30.8–65.27)	28 (21–48)
Alanine aminotransferase (U/L)	23 (15–46.5)	23.68 (17.62–45)	22 (11–52)
Total bilirubin (mg/dL)	0.66 (0.43–2.45)	1.43 (0.61–3.84)	0.56 (0.33–1.28)
Direct bilirubin (mg/dL)	0.35 (0.14–1.91)	0.73 (0.32–2.47)	0.29 (0.12–0.58)
Creatinine (mg/dL)	0.80 (0.50–1.12)	0.90 (0.43–1.39)	0.77 (0.55–1.01)
Blood urea nitrogen (mg/dL)	13 (8–29)	15.5 (9–35)	10.0 (7–22)
Hemoglobin (g/dL)	12.0 (10.1–13.2)	11.7 (9.6–13.2)	12.1 (10.2–13.9)
Hematocrit (%)	35.0 (28.3–40.4)	35.5 (25.1–40.7)	34.6 (31.2–40)
White blood cell count (10^3/μL)	6.18 (4.80–8.00)	6.04 (5.32–8.53)	6.20 (4.6–8)
Platelet count (10^3/μL)	212 (102–294)	166 (102–218)	118 (116–334)
Mean platelet volume (fL)	8.0 (7.2–9)	8.2 (7.3–10.2)	8.0 (7–9)
Platelet mass index	1760.0 (1037.9–2513.5)	1552.5 (1029.2–2196.0)	1920.0 (1242–2679.1)

p=0.019), and platelet count (166 (range: 102-218) versus 118 (range: 116-334) 10^3/μL, p=0.042). On the other hand, according to multivariant analysis there was no statistically significant difference in the terms of headache, blood urea nitrogen, aspartate aminotransferase, total bilirubin, and platelet count. Univariate and multivariate logistic regression analyses of patients with *P. falciparum* and others are presented in Table 2.

Table 2. Univariate and multivariate logistic regression analyses of patients with *P. falciparum* and others*

	Univariate Analysis		Multivariate Analysis	
	OR (95% CI)	p	OR (95% CI)	p
Age, years		0.136		0.546
Age, ≥50 vs. <50	1.123 (0.458–2.752)	0.801		
Gender		0.952		
Headache	0.357 (0.142–0.893)	0.025	0.406 (0.079–2.09)	0.406
Blood urea nitrogen (mg/dL)		0.029	1.029 (0.995–1.06)	0.1
Aspartate aminotransferase (U/L)		0.011	0.995 (0.981–1.01)	0.535
Total bilirubin (mg/dL)		0.019	1.079 (0.926–1.26)	0.328
Hemoglobin (g/dL)		0.171		
Hematocrit (%)		0.870		
White blood cell count (10^3/μL)		0.307		
Platelet count (10^3/μL)		0.042	0.996 (0.989–1)	0.213
Mean platelet volume (fL)		0.276		

*OR – odds ratio; CI – confidence interval

The ROC curve analysis was performed to determine the predictive ability of the blood urea nitrogen, total bilirubin, aspartate aminotransferase, and platelet count for predicting type of malaria. The cut-off values of these parameters according to the best Youden's index, as well as their sensitivity, specificity, AUC, and 95% confidence interval values are presented in Table 3.

Discussion

In current study, we evaluated role of hematological parameters in the differentiation of *P. falciparum* and other plasmodium species. According to the results of this study, there was a statistically significant difference between the *P. falciparum* and other plasmodium species groups for all mentioned parameters.

In our analysis, first, nonparametric comparison tests were used to determine the relationship between scoring systems and mortality. There was significantly difference between groups in the term of headache, blood urea nitrogen, aspartate aminotransferase, total bilirubin, and platelet count. A second analysis was

Table 3. Accuracy of the blood urea nitrogen, total bilirubin, aspartate aminotransferase, and platelet count in predicting type of malaria*

	AUC	Cut–off	Sensitivity	Specificity	+LR	–LR	+PV	–PV	Accuracy	95% CI	p
Blood urea nitrogen (mg/dL)	0.626	≤12	64.3%	59.0%	1.57	0.61	51.9	70.6	61.2	51.1–70.7	0.025
Total bilirubin (mg/dL)	0.684	≤0.61	76.0%	58.1%	1.68	0.44	57.6	73.9	64.3	50.3–76.7	0.009
Aspartate aminotransferase (U/L)	0.656	≤30.8	81.6%	55.6%	1.84	0.33	56.4	81.1	66.3	55.7–89.7	0.007
Platelet count (10^3/μL)	0.619	≤220	53.2%	75.6%	1.62	0.346	76.7	51.7	62.1	52.0–71.5	0.033

*AUC – area under the curve; PV – predictive value; CI – confidence interval; LR – likelihood ratio

performed based on the ROC curve to determine the laboratory parameters' ability to distinguish whether a patient infected with *P. falciparum* or other plasmodium species. AUC values less than 0.5 were evaluated as indistinguishable from random, while those close to 1 were considered close to the perfect model.[7,8] It has been reported that the AUC value should be greater than 0.8 for a model to predict mortality well.[7,8] In the discriminatory power analysis, we determined the AUC value of laboratory parameters lower than 0.7, which was considered to be unacceptable. Thus, our retrospective, comparative study, was demonstrated that laboratory parameters that we investigated were not a predictor of plasmodium species, according to ROC analysis. On the other hand, LRs supply the clearest data on the way in which laboratory parameter can be used reliably. Ratios >5 or < 0.2 provide of strongest evidence.[9,10] In our study group, LR values of laboratory parameters were not in this range. A further analysis was performed based on multivariant analysis. Confirming the ROC analysis results, the multivariate analysis showed that the parameters with a significant difference compared to the univariant analysis result were not sufficient to say that they were statistically independent predictors of the difference between the groups.

Malaria is a disease that is transmitted to humans by the bite of a parasitic mosquito, which can be fatal if not treated in time, and causes fever and chills in seizures. Rare ways of transmission are congenital, blood transfusion, shared injector use, organ transplantation, nosocomial transmission.[11] The simple, effective, and short-term method used in the diagnosis of malaria is the Giemsa stain. In this method, thin smears and thick drops are made from the blood sample taken from the fingertip and after staining with Giemsa, the evolutionary periods of the parasite are searched. If the diagnosis is not made at the first examination, 3 consecutive days of examination are recommended.[12]

Changes in hemoglobin, platelet and leukocyte counts are used to determine the severity of the disease.[13-15] Among the hematological parameters, hemoglobin is the most frequently affected in severe disease. Severe anemia has been reported in 5.5 to 15% of cases. It has been reported that this change may be a change related to geography.[16-18] Platelet count is affected in severe malaria infections, as in sepsis. Disseminated intravascular coagulation, immune mechanisms, and hypersplenism are some examples of mechanisms that could be mechanism of platelet reduction in malaria patients as sepsis and septic shock.[19,20] The transient increase in band cells observed in infection indicates a stronger stimulus for neutrophil production during the acute phase of infection. In the acute phase, premature release of neutrophils from the bone marrow occurs, resulting in an increased proportion of younger, less well-differentiated neutrophils into the circulation. Although this alteration is widely recognized in other acute infectious diseases, few studies have investigated these disorders for neutrophil in malaria infection.[21] Rodrigues-da-Silva et al. compared erythrogram and leucogram of *P. falciparum* and *P. vivax* infections. They evaluated white blood cell counts and red blood cell counts, hemoglobin, hematocrit, reticulocyte, lymphocyte, eosinophil, platelet, segmented neutrophil, band cell, monocyte, and basophil counts. They reported that there was no difference between the *P. falciparum* and *plasmodium vivax* groups in terms of all hematological parameters, except for platelet count.[22] Arévalo-Herrera et al. compared hemoglobin and platelet count of *P. falciparum* and *plasmodium vivax* groups with severe malaria. They found that there was no difference between the two groups in terms of hemoglobin and platelet count. In the current study, it was seen that there was no difference between *P. falciparum* and other *Plasmodium* groups in terms of all hematological parameters.[23] The results of both mentioned studies were compatible with current study. The main difference between the current study and mentioned literature was difference in the methodology of the studies. While thick smear was used as a diagnostic test in both studies, RDT was used in our study. The rapid diagnosis kit could not distinguish between plasmodium types other than *P. falciparum*.

The most important limitation of the current study was its retrospective design. The second limitation is that microscopy was not used to distinguish between *P. falciparum* and other plasmodium species. A third limitation is inability to distinguish other plasmodium species is the inability to perform subgroup analysis. A fourth limitation is that we could not evaluated possible confounders such as bacterial infections. A last limitation is the single-center study and the relatively limited sample size. We suggest that our study results be validated with multicenter studies with larger samples.

Conclusion

The present study was done to test the ability of the hematological parameters to differentiating *P. falciparum* and others. Based on all the observations from the present study, it was concluded that the there was no statistically significant difference between the *P. falciparum* and other *Plasmodium* species groups for all mentioned parameters, total bilirubin, indirect bilirubin, direct bilirubin, creatinine, hemoglobin, platelet count, mean platelet volume, blood urea nitrogen, aspartate transaminase, alanine transaminase, hematocrit, and white blood cell count. These mentioned parameters were found to be not valuable in predicting type of malaria. On the other hand, we recommend confirming the results of our study with larger samples and multicenter studies.

Declarations

Funding

We received no financial support for the research, authorship, or publication of this article.

Author contributions

Conceptualization, S.Ö., A.A, İ.A., and E.A.; Methodology, S.Ö.; Software, E.A.; Validation, S.Ö., A.A, İ.A., and E.A.; Formal Analysis, S.Ö.; Investigation, A.A.; Resources, S.Ö.; Data Curation, A.A.; Writing – Original Draft Preparation, S.Ö.; Writing – Review & Editing, S.Ö.; Visualization, S.Ö.; Supervision, S.Ö., A.A, İ.A., and E.A.; Project Administration, S.Ö., A.A, İ.A., and E.A..; Funding Acquisition, S.Ö., A.A, İ.A., and E.A.

Conflicts of interest

We declare no conflict of interest.

Data availability

The datasets used and/or analyzed during the current study are open from the corresponding author on reasonable request.

Ethics approval

Study was approved by the institutional review board, and a waiver of authorization was given (Ethics Committee decision no. 450, date: 11.22.2021).

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