Original Article

The Association between Parasitemia and Liver Enzyme Alterations in Malaria Patients: ABO Blood Group as A Non-Contributory Factor

Selva Aminizadeh[1], Elham Shokri[1], Mehdi Mohebali[1,2], *Haleh Hanifian[1], Salman Zafari[1], Mahdi Edalati[3], Saeed Bahadory[4], Hossein Hosseinifard[5], *Mehdi Nateghpoor[1]

[1]Department of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
[2]Center for Research of Endemic Parasites of Iran (CREPI), Tehran University of Medical Sciences, Tehran, Iran
[3]Department of Laboratory Sciences, Paramedical Faculty, Tabriz University of Medical Sciences, Tabriz, Iran
[4]Vice-Challenger of Treatment, Imam Hasan Hospital, Alborz University of Medical Sciences, Karaj, Iran
[5]Department of Biostatistics, School of Public Health, Hamadan University of Medical Sciences, Hamadan, Iran

***Corresponding authors:** Dr Haleh Hanifian, E-mail: haleh.hanifian@gmail.com, Prof Mehdi Nateghpour, E-mail: nateghpourm@tums.ac.ir

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Abstract

Background: Malaria, a life-threatening parasitic disease, exhibits diverse clinical manifestations influenced by parasite species, host immunity, and treatment access. Emerging evidence suggests that individual biological factors, such as ABO blood group and liver enzyme status, may also affect disease severity. This study investigates the relationship between blood type, liver enzyme levels, and malaria severity in affected patients**.**

Methods: A case-control study was conducted on malaria patients at the National Malaria Laboratory, Tehran University of Medical Sciences, from May 2022 to October 2024. Blood samples were analyzed for ABO blood grouping and liver enzymes: alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP). Malaria severity was classified according to WHO criteria. Statistical analyses, including chi-square tests and logistic regression, evaluated associations between blood group, liver enzyme levels and disease severity**.**

Results: A total of 100 participants were included: 50 malaria-positive patients and 50 healthy controls, with a mean age of 38.10±16.40 years. Malaria patients showed significantly higher AST, ALT and ALP levels compared to controls ($p= 0.001$). No significant association was found between ABO blood group and liver enzyme levels in either group ($p> 0.05$). Liver enzyme levels correlated significantly with parasitemia ($p< 0.001$), with higher parasite loads linked to greater hepatic dysfunction**.**

Conclusion: This study highlights the significant impact of parasitemia levels on liver function in malaria patients, while the ABO blood group appeared unrelated to liver enzyme alterations. Liver enzyme profiles may serve as valuable biomarkers for assessing malaria severity and guiding clinical management.

Keywords: Malaria; Blood groups; Liver enzyme; Disease severity; Human

Introduction

Despite significant global efforts to prevent and treat malaria, the disease remains a major public health threat, with an estimated 282 million cases and 610,000 deaths reported in 2024 across 80 countries (1). Although both preventable and treatable, malaria continues to impact millions, particularly in endemic regions. In Iran, the disease persists in the southern and southeastern provinces of Sistan and Baluchestan and Hormozgan, where over 80% of the country's malaria cases are documented (2). Malaria in humans is caused by five *Plasmodium* species, *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi*, transmitted by

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the bite of infected female *Anopheles* mosquitoes (3, 4). Following transmission, sporozoites are injected into the skin during the mosquito's blood meal, where they may persist for 1–3 hours before potentially being cleared by the lymphatic system, initiating an immune response. Surviving sporozoites enter the bloodstream, rapidly migrating to the liver, where they infect hepatocytes and transform into exoerythrocytic forms over 2–10 days. This process results in the release of up to 40,000 merozoites into the bloodstream via merosomes. Although the liver stage is clinically silent, it represents a crucial focus for research as it precedes the symptomatic blood-stage infection and progression to severe disease (5–8). Jaundice and liver dysfunction are common clinical features in severe malaria cases (9, 10). Histopathological examination of affected liver tissue typically reveals hepatocyte necrosis, granulomatous lesions, Kupffer cell hyperplasia, malarial pigment deposition, bile flow obstruction and monocyte infiltration within malarial nodules. These pathological changes contribute to an increased risk of liver failure and systemic complications in severe malaria (9, 11–13). Although clinical malaria is frequently associated with liver abnormalities, comprehensive knowledge about its broader clinical aspects remains limited. In most of the literature, malaria-related liver dysfunction is primarily characterized by abnormal laboratory values, particularly elevated levels of bilirubin, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (13–16). Damage to hepatocytes leads to the leakage of intracellular enzymes such as AST, ALT and alkaline phosphatase (ALP) into the bloodstream, resulting in elevated serum levels of these enzymes in malaria patients (4). Monitoring changes in these biomarkers between the acute and convalescent phases may aid in evaluating disease progression and the early response to treatment (17, 18). Additionally, variations in the physicochemical properties of *Plasmodium*-infected blood appear to depend on factors such as malaria endemicity, nutritional status, demographic characteristics and individual immune responses (19). The ABO blood group system is genetically determined and varies across populations and ethnic groups (20). These carbohydrate-based antigens are present on red blood cells and various tissues, influencing protein function and cell-to-cell interactions, especially during infections. In malaria, parasite adhesion to host cells contributes to disease severity and these interactions may differ based on blood group (21, 22). Beyond host physiological responses, genetic factors such as the ABO blood group may also influence disease occurrence. Some experimental and epidemiological studies have shown differences in the propensity of *Anopheles* mosquitoes to feed on ABO blood groups, as well as the incidence of malaria in different blood groups (23, 24). Therefore, this study aimed to investigate the potential association between ABO blood group, liver function test abnormalities, and parasite levels in patients with *P. falciparum*, *P. vivax*, and mixed-species infections in an endemic region of Iran.

Materials and Methods

Study design and participants

A case-control study was conducted at the National Malaria Laboratory, Tehran University of Medical Sciences, from May 2022 to October 2024. A total of 100 participants were enrolled, including 50 malaria-positive patients and 50 healthy controls. The control group in this study consisted of individuals selected from the same source population as the case group and matched in age, sex and geographic region. Malaria infection was confirmed negative using peripheral blood smear and rapid diagnostic test (RDT). Exclusion criteria included individuals with a history of chronic liver disease, hepatitis B surface antigen (HBs Ag) or hepatitis C virus antigen (HCV

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Ag) positivity, and other known infectious or non-infectious causes of liver dysfunction.

Sample collection and laboratory analysis

Blood samples were collected from all participants using standard phlebotomy techniques. ABO blood grouping was performed using both the cell grouping (forward type) and serum grouping (reverse type) methods to ensure accuracy and consistency. Liver function tests (LFTs), e.g., aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP), were measured using routine automated biochemistry analyzers based on standard enzymatic colorimetric methods using the Delta Darman Part kit (Tehran, Iran). To exclude other causes of liver dysfunction, all participants were screened for hepatitis B surface antigen (HBs Ag) and hepatitis C virus antigen (HCV Ag) using the Wantai kit (Beijing, China) according to the manufacturer's instructions.

Parasitemia and species identification

Malaria diagnosis was confirmed by examining both thin and thick peripheral blood smears, prepared from capillary blood samples. Smears were stained with 10% Giemsa solution for 20 minutes, then examined under a light microscope. Parasite species identification (*P. falciparum*, *P. vivax*, or mixed infections) was performed on thin smears, while parasitemia levels were calculated from thick smears by counting the number of asexual parasites per 200 white blood cells (WBCs) and converting the result to parasites per microliter of blood, assuming a standard WBC count of 8,000/µL. Parasitemia was categorized as few (1 to 999 parasites per microliter), moderate++ (1000 to 9999 parasites per microliter), or many+++ (over 10,000 parasites per microliter) (25).

Classification of malaria severity

Malaria severity was classified according to the World Health Organization (WHO) criteria. Severe malaria was defined by the presence of one or more of the following features: impaired consciousness or coma (cerebral malaria), severe anemia (hemoglobin <5 g/dL), hyperparasitemia (parasite density ≥100,000 parasites/µL), jaundice accompanied by evidence of organ dysfunction, respiratory distress, or signs of vital organ failure. Patients who did not meet these criteria were classified as having uncomplicated malaria.

Statistical analyses

The results were reported in the form of descriptive statistics as mean (standard deviation) for quantitative variables and number (percentage) for qualitative variables. In order to compare the frequency distribution of gender and blood group between the two diseased and healthy groups, the Chi-square test was used. The variables of origin, parasitemia, and parasite species were measured only in the diseased group; only the frequency of these variables in the diseased group was reported. In order to compare liver enzymes between the two groups, the independent t-test was used. A probability value (p-value) less than 5 percent was considered a significant level and R software version 4.4.2 was used to analyze the data.

Results

Participant demographics

A total of 100 participants were enrolled in the study, comprising 50 malaria-positive patients and 50 healthy controls. Among the total participants, 21 were female and 79 were male. The age range was between 12 and 72 years, with a mean age of 38.10±16.40 years. There was a statistically significant difference in gender distribution between the patient and control groups (p= 0.007), with females representing 10% of the patient group and 32% of the control group. The nationality distribution among malaria patients was heterogeneous, including 44% Iranian, 34% Afghan, 4% Indian, 4% Pakistani and 2% each from Cameroon, Sierra Leone, Sudan and Kenya/Somalia. All healthy controls were Iranian nationals (Table 1).

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Parasitemia levels and parasite species

In this study, parasitemia levels among the malaria-positive patients were categorized into three distinct groups based on the density of parasites observed in peripheral blood smears. Approximately 10% of the patients had a low parasite density (few), indicating a mild level of infection. The majority of patients, 68%, exhibited a moderate parasitemia, reflecting a more substantial burden of parasites circulating in the bloodstream. Lastly, 22% of the patients were classified as having a high parasite density (many), which is often associated with more severe clinical manifestations and a greater risk of complications.

Regarding the species distribution of the malaria parasites, the vast majority of infections were caused by *P. vivax*, accounting for 76% of the cases. In contrast, infections due to *P. falciparum* represented 24% of the total malaria cases (Fig. 1). This species distribution aligns with regional epidemiological patterns observed in Iran and neighboring endemic areas, where *P. vivax* is the predominant malaria species, but *P. falciparum* remains a significant cause of morbidity due to its higher pathogenicity.

Liver enzyme levels and blood group

The mean levels of liver enzymes-AST, ALT and ALP-were significantly elevated in malaria patients compared to healthy controls ($p< 0.001$ for all enzymes). Specifically, among patients, mean AST levels varied by blood group, ranging from 96.24±34.49 IU/L in group AB to 158.81±76.98 IU/L in group B, whereas controls exhibited consistently low AST levels of 30.93±9.04 to 31.37±7.83 IU/L across blood groups. Similar patterns were observed for ALT and ALP, with malaria patients showing markedly higher enzyme levels than controls, regardless of blood group (Table 2).

Based on Table 3, it can be seen that with blood group, gender and age remaining constant, the average AST in the patient group was 104.71 units lower than the healthy group, which was statistically significant ($p< 0.001$). Also, the average ALT in the patient group was 90.53 units lower than the healthy group, which was also statistically significant ($p< 0.001$) and the average ALP in the patient group was 265.35 units lower than the healthy group, which was also statistically significant ($p< 0.001$).

Table 4 shows the results of multivariate regression to investigate the relationship between blood group and liver enzyme levels. According to Table 4, it is observed that the effect of parasitemia and gender on AST level was significant, but the effect of age on AST was not significant. On average, the AST level in patients with blood groups A and AB was 12.51 and 13.02 units lower than in patients with blood group O, respectively, but was 19.56 units higher in patients with blood group B. These values were not statistically significant.

Statistical analysis revealed no significant effect of ABO blood group on liver enzyme levels within either the patient or control groups (p-values: AST=0.027, ALT=0.033, ALP=0.039), indicating that the observed liver enzyme elevations are primarily associated with malaria infection rather than blood group differences (Fig. 2).

Liver enzyme levels and parasitemia

Liver enzyme levels varied significantly according to parasitemia intensity ($p< 0.001$ for AST, ALT and ALP). Patients with many parasites demonstrated the highest mean enzyme levels (AST: 196.34±65.15 IU/L; ALT: 174.45± 57.48 IU/L; ALP: 633.73±168.04 IU/L), followed by those with moderate parasitemia and the lowest levels were observed in patients with few parasites (AST: 74.55±28.55 IU/L; ALT: 62.91±24.30 IU/L; ALP: 324.20±54.55 IU/L) (Table 5). These findings suggest a positive correlation between parasite density and hepatic dysfunction.

Table 6 shows the results of post hoc tests using Tukey's method for pairwise comparison of groups. According to the results, it can be seen that the difference in mean liver enzyme levels between each pair of parasitemia levels was statistically significant.

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Table 7 shows the results of the regression model to compare the levels of liver enzymes between the two species, controlling for gender and age in the patients. Based on the results, it is observed that the mean levels of liver enzymes AST, ALT and ALP in patients with *P. vivax* were 42.70, 34.17 and 54.78 units lower than in patients with *P. falciparum*, respectively. These values were statistically significant for AST and ALT ($p< 0.05$) but not for ALP ($p> 0.05$).

Spearman's rank correlation was calculated between parasitemia and liver enzyme levels. The correlation coefficient between parasitemia and liver enzyme levels of AST, ALT and ALP was found to be 0.58, 0.60 and 0.55, respectively, which were statistically significant ($p< 0.001$).

Table 1. Demographic characteristics of study participants

		Groups			*p*-value
		Patient *n* (%)	**Healthy *n* (%)**	**Total *n* (%)**	
Gender	Female	5 (10)	16 (32)	21 (21)	0.007
	Male	45 (90)	34 (68)	79 (79)	
Origin	Iran	22 (44)	50 (100)	72 (72)	-
	Afghanistan	17 (34)	-	17 (17)	
	India	2 (4)	-	2 (2)	
	Kameron	1 (2)	-	1 (1)	
	Kenya and Somalia	1 (2)	-	1 (1)	
	Pakistan	2 (4)	-	2 (2)	
	Sierra Leone	1 (2)	-	1 (1)	
	Sudan	1 (2)	-	1 (1)	
	NA	3 (6)	-	3 (3)	
Parasitemia	Few	5 (10)	-	5 (10)	-
	Many	11 (22)	-	11 (22)	
	Moderate	34 (68)	-	34 (68)	
Parasite species	*P. f*	12 (24)	-	12 (24)	-
	P. v	38 (76)	-	38 (76)	

NA: Not stated, *n*: number, *P. f*: *Plasmodium falciparum*, *P. v*: *Plasmodium vivax*

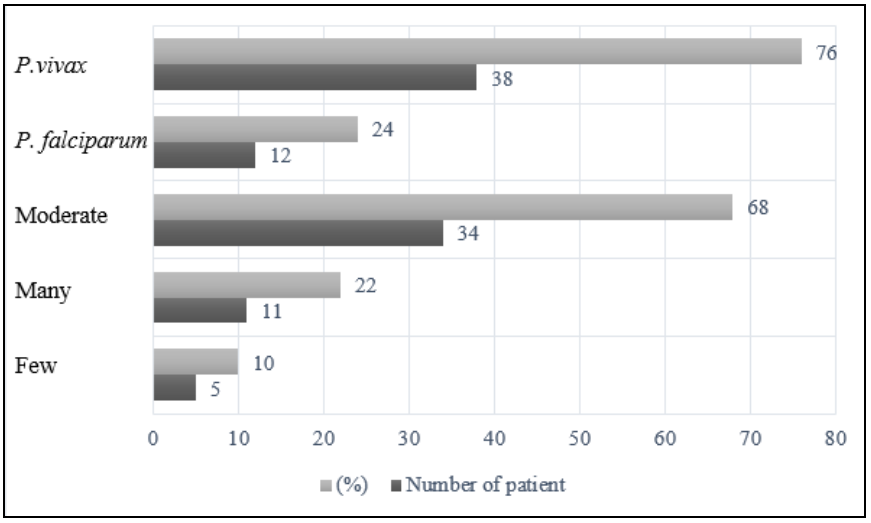

Fig. 1. Distribution of *Plasmodium* species (*Plasmodium falciparum*, *P. vivax*) and parasitemia levels (few, moderate, and many) among malaria patients

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Table 2. Liver enzyme (AST, ALT and ALP) levels (Mean±SD) by ABO blood group in malaria patients and healthy controls

Enzyme	ABO	Patients *n*	Mean±SD (IU/L)	Controls *n*	Mean±SD (IU/L)	*p*-value (group)	*p*-value (blood group)*
AST	A	17	123.47±42.82	11	31.37±7.83	<0.001	0.027
	AB	6	96.24±34.49	6	31.25±6.19		
	B	16	158.81±76.98	13	30.93±9.04		
	O	11	138.79±48.92	20	31.14±8.22		
ALT	A	17	109.62±39.49	11	25.32±9.27	<0.001	0.033
	AB	6	87.78±33.11	6	30.94±6.08		
	B	16	139.20±69.37	13	31.08±9.69		
	O	11	118.76±42.23	20	24.99±8.20		
ALP	A	17	448.88±134.36	11	226.91±87.00	<0.001	0.039
	AB	6	417.67±114.18	6	236.67±65.45		
	B	16	570.19±188.74	13	234.23±65.13		
	O	11	500.18±118.88	20	237.60±51.86		

n: number, AST: aspartate aminotransferase, ALT: alanine aminotransferase and ALP: alkaline phosphatase, *: The reported p-values are for the General linear model (GLM)

Table 3. Estimated regression coefficients based on the General Linear Model (GLM)

	Variables	B	Std. Error	t-value	p-value	95% Confidence Interval
AST	Intercept	16.955	15.243	1.112	0.269	(-13.31; 47.23)
	Healthy				Ref	
	Patient	-104.709	9.186	-11.398	<0.001	(-122.95; -86.47)
	O				Ref	
	A	-9.178	10.632	-0.863	0.390	(-30.29; 11.94)
	AB	-20.410	13.689	-1.491	0.139	(-47.59; 6.77)
	B	17.277	10.646	1.623	0.108	(-3.86; 38.42)
	Female				Ref	
	Male	19.130	10.911	1.753	0.083	(-2.54; 40.8)
	Age	0.465	0.271	1.716	0.090	(-0.07; 1)
ALT	Intercept	15.818	13.803	1.146	0.255	(-11.59; 43.23)
	Healthy				Ref	
	Patient	-90.538	8.319	-10.884	<0.001	(-107.06; -74.02)
	O				Ref	
	A	-5.526	9.628	-0.574	0.567	(-24.65; 13.59)
	AB	-11.577	12.396	-0.934	0.353	(-36.19; 13.04)
	B	19.290	9.641	2.001	0.048	(0.15; 38.43)
	Female				Ref	
	Male	17.147	9.880	1.736	0.086	(- 2.47; 36.77)
	Age	0.335	0.246	1.365	0.176	(-0.15; 0.82)
ALP	Intercept	205.536	44.361	4.633	<0.001	(117.44; 293.63)
	Healthy				Ref	
	Patient	-265.358	26.734	-9.926	<0.001	(-318.45; -212.27)
	O				Ref	
	A	-36.907	30.943	-1.193	0.236	(-98.35; 24.54)
	AB	-41.611	39.838	-1.044	0.299	(-120.72; 37.5)
	B	47.293	30.983	1.526	0.130	(-14.23; 108.82)
	Female				Ref	
	Male	33.509	31.753	1.055	0.294	(- 29.55; 96.56)
	Age	0.923	0.789	1.170	0.245	(-0.64; 2.49)

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Table 4. Multivariate regression coefficients for examining the relationship between blood type and liver enzyme levels

Liver enzyme levels	Variables		Unstandardized Coefficients	Std. Error	Standardized Coefficients	t-value	p-value
	Intercept		-23.66	19.80		-1.19	0.235
		O			Ref		
	Blood	A	-12.51	10.02	-0.08	-1.25	0.215
	Group	AB	-13.02	13.01	-0.06	-1.00	0.320
		B	19.56	10.02	0.13	1.95	0.054
AST		Few			Ref		
	Parasitemia	Moderate	137.71	12.46	0.67	11.05	<0.001
		Many	90.00	9.51	0.66	9.46	<0.001
	Gender	Female			Ref		
		Male	25.23	10.38	0.15	2.43	0.017
	Age		0.27	0.26	0.07	1.03	0.307
	Intercept		-20.41	18.17		-1.12	0.264
		O			Ref		
	Blood	A	-8.26	9.20	-0.06	-0.90	0.371
	Group	AB	-5.51	11.93	-0.03	-0.46	0.645
		B	21.17	9.19	0.16	2.30	0.023
ALT		Few			Ref		
	Parasitemia	Moderate	117.62	11.43	0.65	10.29	<0.001
		Many	78.47	8.72	0.65	8.99	<0.001
	Gender	Female			Ref		
		Male	22.15	9.52	0.15	2.33	0.022
	Age		0.17	0.24	0.05	0.72	0.472
	Intercept		133.55	59.59		2.24	0.027
		O			Ref		
	Blood	A	-43.96	30.17	-0.11	-1.46	0.148
	Group	AB	-25.98	39.15	-0.05	-0.66	0.509
		B	52.13	30.14	0.13	1.73	0.087
ALP		Few			Ref		
	Parasitemia	Moderate	335.16	37.49	0.62	8.94	<0.001
		Many	234.25	28.62	0.65	8.19	<0.001
	Gender	Female			Ref		
		Male	46.40	31.23	0.11	1.49	0.141
	Age		0.50	0.78	0.05	0.64	0.521

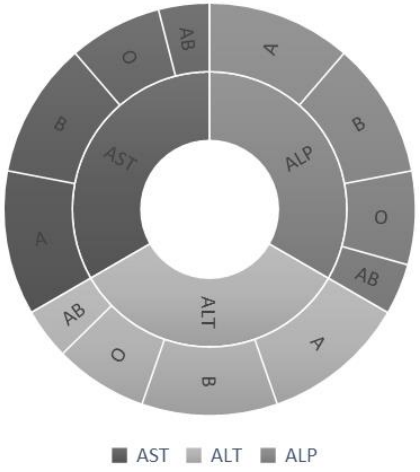

Fig. 2. The effect of ABO blood group on liver enzyme levels (AST, ALT and ALP)

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Table 5. Liver enzyme (AST, ALT and ALP) levels (Mean ± SD) by parasitemia intensity in malaria patients

Enzyme	Parasitemia level	Mean ± SD (IU/L)	*p*-value
AST	Few	74.55 ± 28.55	<0.001
	Moderate	123.87 ± 42.29	
	Many	196.34 ± 65.15	
ALT	Few	62.91 ± 24.30	<0.001
	Moderate	108.54 ± 37.19	
	Many	174.45 ± 57.48	
ALP	Few	324.20 ± 54.55	<0.001
	Moderate	475. 59 ± 126.47	
	Many	633.73 ± 168.04	

AST: aspartate aminotransferase, ALT: alanine aminotransferase and ALP: alkaline phosphatase

Table 6. Post hoc test results for pairwise comparisons between parasitemia levels

Dependent Variable	Group I	Group J	Mean Difference (I-J)	Std. Error	95% CI for Mean Difference	p-value
AST	Few	Many	-121.79	25.46	(-173.01; -70.57)	<0.001
		Moderate	-49.33	22.61	(-94.81; -3.84)	0.034
	Many	Moderate	72.47	16.37	(39.52; 105.41)	<0.001
ALT	Few	Many	-111.54	22.40	(-156.6; -66.48)	<0.001
		Moderate	-45.63	19.89	(-85.64; -5.62)	0.026
	Many	Moderate	65.91	14.40	(36.93; 94.89)	<0.001
ALP	Few	Many	-309.53	71.33	(-453.03; -166.02)	<0.001
		Moderate	-151.39	63.35	(-278.83; -23.95)	0.021
	Many	Moderate	158.14	45.88	(65.85; 250.43)	0.001

Table 7. Results of the regression model to compare liver enzyme levels between *Plasmodium falciparum* and *P. vivax*, controlling for gender and age in patients

			Unstandardized Coefficients	Std. Error	Standardized Coefficients	t-value	p-value
AST	Intercept		118.712	71.023		1.671	0.101
	Parasite species	*P. falciparum*			Ref		
		P. vivax	-42.703	18.354	-0.314	-2.327	0.024
	Gender	Female			Ref		
		Male	55.004	25.187	0.284	2.184	0.034
	Age		0.906	0.593	0.204	1.526	0.134
ALT	Intercept		91.971	64.401		1.428	0.160
	Parasite species	*P. falciparum*			Ref		
		P. vivax	-34.173	16.643	-0.282	-2.053	0.046
	Gender	Female			Ref		
		Male	50.845	22.839	0.294	2.226	0.031
	Age		0.743	0.538	0.188	1.381	0.174
ALP	Intercept		459.271	200.484		2.291	0.027
	Parasite species	*P. falciparum*			Ref		
		P. vivax	-78.548	51.810	-0.217	-1.516	0.136
	Gender	Female			Ref		
		Male	90.356	71.099	0.176	1.271	0.210
	Age		2.487	1.675	0.211	1.485	0.144

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Discussion

The present study provides compelling evidence of significant hepatic involvement in malaria patients, demonstrated by marked elevations in serum AST, ALT and ALP levels compared to healthy controls. These findings confirm the hepatic tropism of *Plasmodium* species during the erythrocytic stage and underscore the importance of routine liver function monitoring in malaria management, particularly in endemic regions like Iran, where *P. vivax* predominates (2). Importantly, the significant liver injury observed in our predominantly *P. vivax* cohort, which included both Iranian and non-Iranian nationals, adds population-specific evidence to the growing body of literature challenging the traditional view of *P. vivax* malaria as a benign infection. This demographic heterogeneity reflects the current epidemiology of malaria in Iran and underscores that the capacity of *P. vivax* to induce clinically relevant hepatic dysfunction is not restricted to a particular nationality or host background. Our findings therefore support the concept that hepatic complications are a fundamental component of malaria pathophysiology in *P. vivax* infections, rather than an exception limited to *P. falciparum*. These findings are particularly relevant for regions such as Iran, where *P. vivax* predominates and is often under-recognized as a cause of severe systemic involvement (9, 10, 26). These results mirror findings from Kochar et al. (13) and Jain et al. (9), both of whom demonstrated hepatic dysfunction even in *P. vivax* infections previously considered benign. This paradigm shift is critical for clinicians and public health policymakers in *P. vivax*-endemic areas. The significant liver injury observed in our predominantly *P. vivax* cohort, which included both Iranian and non-Iranian nationals, adds to the growing body of evidence challenging the traditional benign classification of this species. This reinforces that hepatic complications are a core feature of malaria pathophysiology, not an exception reserved for *P. falciparum*. Our study reveals a strong, positive and statistically significant correlation between parasitemia intensity and the degree of liver enzyme derangement. Patients with high parasitemia exhibited enzyme levels more than threefold greater than those with low parasite densities, a pattern also reported by Kochar et al. (13) and Abro et al. (16). These findings highlight parasitemia as not merely a diagnostic parameter but a reliable prognostic marker of hepatic involvement, which should be closely monitored to pre-empt complications such as malarial hepatitis, cholestasis, or even acute liver failure (12–14). Interestingly, no significant association was observed between ABO blood group and liver enzyme alterations in either malaria patients or healthy controls. While ABO blood group antigens have been implicated in malaria pathogenesis through mechanisms such as rosetting, cytoadherence and modulation of parasitemia thresholds, our findings suggest that these effects may not extend to hepatic involvement (20, 21). This implies that malaria-associated liver dysfunction is more directly driven by parasite burden and host inflammatory responses than by erythrocyte surface antigens. In other words, although the ABO blood group may influence susceptibility to infection or certain hematological complications, it does not appear to play a determining role in malaria-induced hepatopathy. This distinction is clinically relevant, as it supports the use of liver enzyme abnormalities as universal severity markers across blood groups, without the need for blood group-specific risk stratification. Our findings align with Gupte et al. (20) and Amodu et al. (22), indicating that ABO antigens may not influence hepatic involvement directly, even if they modulate other clinical outcomes like anemia or parasitemia thresholds. This distinction clarifies the need for further focused research on the immunogenetic determinants of malarial hepatopathy beyond ABO grouping. The observed distribution of parasite species, predominantly *P.*

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vivax (76%) with a smaller proportion of *P. falciparum* (24%), aligns with regional malaria epidemiology in southern Iran (2). However, the substantial hepatic involvement in *P. vivax* cases underscores the evolving clinical profile of this species. Growing international evidence suggests that *P. vivax* malaria, particularly in Southeast Asia and the Eastern Mediterranean region, is increasingly associated with severe hepatic complications (10, 11, 13, 26), likely driven by parasite genetic shifts, host factors and regional treatment delays. This study's strengths include its prospective, controlled design, standardized parasitological and biochemical assessments and strict exclusion of confounding hepatopathies such as viral hepatitis through ELISA screening. Nevertheless, certain limitations merit acknowledgment. The modest sample size, particularly within blood group and parasite species subgroups, may limit the generalizability of the null association findings and constrain statistical power for stratified analyses. Additionally, the cross-sectional design limits assessment of dynamic changes in liver enzymes during treatment and convalescence. Longitudinal studies are necessary to elucidate recovery patterns, relapse risk in *P. vivax* and the long-term hepatic sequelae of malaria infections. Clinically, these findings reinforce the necessity for comprehensive liver function monitoring in malaria patients, particularly those with high parasitemia. Elevated AST, ALT and ALP levels are valuable markers for anticipating complications and should inform management decisions such as hospitalization, adjunctive therapies and hepatic protective strategies. In addition, future studies with a larger sample size are recommended to make the results more accurate. Furthermore, the demographic differences between our patient and control groups, particularly in gender and nationality, though reflective of the local malaria burden, may introduce confounding. Future studies with matched controls would help isolate the specific effects of malaria infection.

Conclusion

This study contributes novel regional data on the relationship between malaria, parasitemia and hepatic dysfunction in Iran, supporting global evidence that *P. vivax* can no longer be considered a purely benign infection. Future multicenter, longitudinal and molecular studies should aim to delineate the precise pathophysiological mechanisms of hepatic injury in both *P. falciparum* and *P. vivax* malaria, potentially integrating host immunogenetic profiles and parasite virulence factors to refine prognostic models and clinical care pathways in endemic settings.

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Ethics considerations

This study was approved by the ethics committee of Tehran University of Medical Sciences (IR.TUMS.SPH.REC.1404.276).

Conflict of interest statement

The authors have declared that no competing interests exist.

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