IJMPES International Journal of
Medical Parasitology & Epidemiology Sciences

http://ijmpes.com
doi 10.34172/ijmpes.5190
2025;6(1):11-19
eISSN 2766-6492

Original Article

TNF-α 238 Alleles Polymorphism and its Association With TNF-α Levels in the Severe Malaria Anemia Among Sudanese Children

Khalid Abdelsamea Mohamedahmed[1,2*], Bakri Yousif Mohammed Nour[3], Mohamed Youisf Mohamed Elshiekh[3], Adam Dawoud Abakar[3], Yousef Gharedaghi[4], Salah Eldin G. Elzaki[5], Yagoob Garedaghi[6*], Roa Osman Elsumani Koko[7]

[1]Department of Hematology and Immunology, Faculty of Medical Laboratory Sciences, University of Gezira, Wad Medani, Sudan
[2]Department of Medical Laboratory Sciences, Faculty of Applied Medical Sciences, Jerash University, Jerash, Jordan
[3]Department of Medical Parasitology, Faculty of Medical Laboratory Sciences, University of Gezira, Wad Medani, Sudan
[4]Department of Basic Sciences, Faculty of Medicine, Baqiyatallah University of Medical Sciences, Tehran, Iran
[5]Department of Epidemiology, Tropical Medicine Research Institute, National Centre for Research, Khartoum, Sudan
[6]Department of Parasitology, Faculty of Veterinary Medicine, Tabriz Medical Sciences, Islamic Azad University, Tabriz, Iran
[7]Department of Molecular Biology, Faculty of Medical Laboratory Sciences, University of Gezira, Wad Medani, Sudan

Abstract

Introduction: Tumor necrosis factor alpha (TNF-α) levels overproduction and promoter polymorphisms at TNF-α 238 alleles may play central role in reduced red cell production and malaria-related anemia through suppression of bone marrow erythropoiesis and dyserythropoiesis. This study aimed to evaluate the TNF-α 238 allele's polymorphism and its association with TNF-α levels in the children with falciparum malaria.

Methods: A longitudinal hospital-based study was conducted among 100 children with severe falciparum malaria (mean age 8.63 ± 3.40 years) and 100 children with uncomplicated falciparum malaria (mean age 8.83 ± 4.20 years). TNF-α level was measured using Human TNF-α ELISA MAX™ Deluxe Sets. PCR was used for detecting TNF-α 238 allele's polymorphism. Obtained data were analyzed by SPSS (Version 20.0) and StatDisk (Version 13.0).

Results: TNF-α 238A allele was a common allele (66.8%). Falciparum malaria-related anemia accounted for 32%, commonly in severe malaria (SM) (55%) compared to uncomplicated malaria (UM) (9%) ($P=0.000$). Otherwise, the average of TNF-α levels strongly positively correlated with the severity of anemia (r + 0.309; $P=0.000$). The TNF-α 238 A allele accounts for 83.6% of malaria anemia ($P=0.000$) and 100% severe anemia ($P=0.000$).

Conclusion: Overproduction of TNF-α is essential for the elimination and clearance of falciparum parasite but may be associated with severity of malaria and malaria anemia. Overproduction of TNF-α in children with TNF-α 238 A allele may result in falciparum malaria-related anemia among children. These findings will assist clinicians in better managing severe malaria-related anemia cases.

Keyword: Falciparum malaria anemia, TNF-α levels, TNF-α 238 alleles, RBCs parameters, Sudanese children

Received: August 13, 2024, **Accepted:** January 19, 2025, **ePublished:** March 29, 2025

Introduction

Malaria is a human intracellular protozoan parasitic disease caused by female anopheline mosquitoes inoculated genus *Plasmodium* parasite (*P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale*, and *P. knowlesi*) (1). Falciparum malaria is still a major health problem in Sudan accounts for up to 80% of malaria cases globally (2) and about 87.6% of malaria cases in Sudan (3,4). The disease is becoming more prevalent due to poor sanitation and a lack of strong preventive factors. Children suffer more malaria episodes and are more prone to severe malaria compared to adults and accounted for 61% (266 000) of all malaria deaths. In fact, about 28 500 children died before their fifth birthdays in 2016 in Africa (2). Falciparum malaria may result in variable clinical symptoms, ranging from very mild symptoms to severe disease and even death (4). Falciparum malaria can be categorized in two groups: uncomplicated or complicated (severe) (5). The classical uncomplicated malaria (UM) has three stages (cold stage, a hot stage, and a sweating stage) (6). If falciparum malaria is not treated properly may occur the following complications: cerebral malaria, severe anemia, hemoglobinuria, pulmonary edema, thrombocytopenia, cardiovascular collapse, shock, kidney failure, hyperparasitemia, metabolic acidosis and hypoglycemia (6). Falciparum malaria is linked to a number of hematological abnormalities involving the major blood cell types, including red blood cells, white blood cells, and platelets (7,8), all of which play factors in the disease's severity. Malaria-related anemia can result in death, especially among vulnerable populations such as children. WHO defines mild anemia as a hemoglobin (Hb) of between 10 g/dL and 10.9 g/dL, moderate anemia

*Corresponding Authors: Khalid Abdelsamea Mohamedahmed, Email: khalid.gu89@gmail.com; Yagoob Garedaghi, Email: yagoob.garedaghi@gmail.com

as between 7 g/dL and 9.9 g/dL, and severe anemia as below 7 g/dL; A surveys conducted in 16 African countries between 2015 and 2017 showed that the prevalence among young children with positive for malaria showed anemia was 79%, mild anemia 21%, moderate anemia 50% and severe anemia 8% (2). The first-line defense against malaria is innate immune cells and their cytokines (Tumor necrosis factor alpha [TNF-α], interleukin 12 [IL-12], interferon gamma [IFN-γ], and nitric oxide [NO]), and second line is adaptive immune response primarily depends on the actions of the α/β T cells (CD + 4, CD + 8) and the B cells (9). TNF-α is a common proinflammatory cytokine. TNF-α is plays a central role in malaria pathogenicity either in the cure or complication of malaria. Their high level and is associated with severe falciparum malaria and is equivocal. Polymorphisms in the TNF-α gene have been associated with increased susceptibility to severe malaria (10-12). Many studies have detected the TNF SNPs at TNF-α 238 exhibit differential associations to malaria and TNF-α production in different populations (13-17). TNF-α levels overproduction and promoter polymorphisms at TNF-α 238 alleles may play a central role in reduced red cell production and malaria-related anemia through suppression of bone marrow erythropoiesis and dyserythropoiesis (18). The purpose of this study was to compare and correlate TNF-α levels and TNF-α 238 alleles polymorphism (A allele/ G allele) between falciparum malaria severity and malaria anemia severity among Sudanese children with falciparum malaria.

Material and Methods

Study Design, Area, and Population

A longitudinal hospital-based study was conducted among 200 Sudanese children at Wad Medani Pediatric Hospital, Gezira State, Sudan from November 2016 to June 2019. 100 children were previously diagnosed with severe falciparum malaria by blood film and WHO criteria, and 100 children were previously diagnosed with uncomplicated falciparum malaria by blood film or thin blood-smear (19).

Inclusion Criteria

The study included sick children with falciparum malaria aging 1 month to 18 years old, from both genders, and residing in Gezira state who were admitted to Wad Medani Pediatric Teaching Hospital (Figure 1).

Exclusion Criteria

Sick children with mixed malaria or vivax malaria were excluded from this study. Those aging ≥ 18 years old, those residing outside Gezira State and those suffering from a recent infection, malignancy, and thrombosis, and those on anticoagulant and anti-inflammatory medication were also excluded.

Define Uncomplicated and Severe Falciparum Malaria

Falciparum Malaria can be categorized in two groups: uncomplicated or complicated (severe) (5).

The classical UM has three stages (cold stage, a hot stage, and a sweating stage) diagnosed by blood film or ICT and clinical findings (6,19).

Severe falciparum malaria diagnosed by blood film and WHO criteria (presence of 2 or more the following complications: cerebral malaria, severe anemia, hemoglobinuria, pulmonary edema, thrombocytopenia, cardiovascular collapse, shock, kidney failure, hyperparasitemia, metabolic acidosis and hypoglycemia) (6,19).

Sample Collection and Preparation

Four milliliters venous blood sample was collected by clean

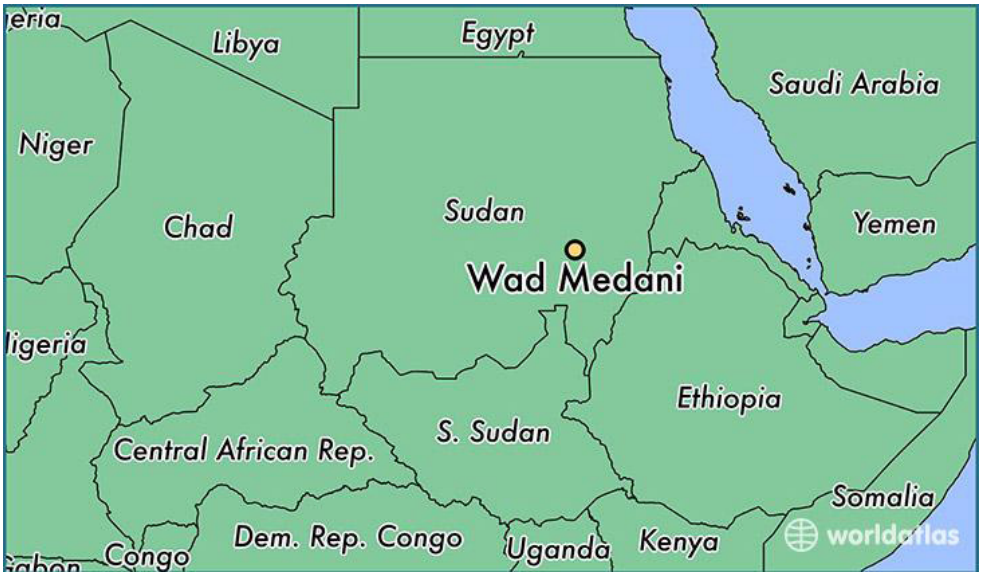

Figure 1. Location of Wad Medani City, Gezira State, Sudan (World Atlas, 2015)

venipuncture for all patients. 2 mL in a plain container and 2 mL in EDTA container. Thin and thick films were prepared immediately. Serum was obtained immediately after blood collection by blood centrifugation of plain container at 1200 rpm for 10 minutes (20). Measurement of red blood cell (RBC) parameters and DNA extraction were performed using samples from the EDTA container.

Measurement of RBCs Parameters

RBCs parameters (RBCs count, Hb g/dL, PCV %, MCV fl, MCH pg, MCHC g/L) were determined using the Sysmex XP-300 N automated hematology analyzer (Sysmex, Kobe, Japan).

Define Anemia and the Severity of the Anemia

Anemia was defined as Hb of less than 12 g/dL (19). WHO defines mild anemia as a Hb of between 10 g/dL and 10.9 g/dL, moderate anemia as between 7 g/dL and 9.9 g/dL, and severe anemia as below 7 g/dL (2).

TNF-α Level Measurement

ELISA was further processed for TNF-α level from serum sample using Human TNF-α ELISA MAX™ Deluxe Sets (BioLegend, Inc).

DNA Extraction

DNA extraction was done using G-DEX™ IIb Genomic DNA Extraction Kit. The extracted DNA concentration was measured by reading the absorbance at 260 nm using a nanosystem. An absorbance ratio of 260 nm and 280 nm gives an estimate of the purity of the solution (DNA product concentration: 5 µg/L; DNA product purity: 1.68). All samples were store at -20 °C till PCR amplification.

TNF-α 238 Alleles Polymorphism Analysis

PCR and gel running system were used for detecting TNF-α 238 Alleles polymorphism (A allele/ G allele). PCR was done to detect TNF-α 238 alleles polymorphism using conserved primer pairs (Macrogen, Korea) (Common TNF "CCGGATCATGCTTTCAGTGC"; TNF 238A allele "AGACCCCCCTCGGAATCG"; and TNF 238G allele "AAGACCCCCCTCGGAATC") to generate 459- and 460-bp products (13). Common TNF-α primer was prepared by adding 300 µL deionized sterile water, TNF-α 238A allele and 238G allele primers were prepared by adding 320 µL D.W. Each of the primers was prepared as follows: 10µL of each stock primer (100 µM) were added to 90 µL PCR water (Deionized sterile water) and aliquoted in 0.5 ml PCR polypropylene tube to yield a concentration of 10µM, and the solution was mixed.

PCR reaction contains PCR master mix (APS LABS, India), Common TNF-α primer, TNF-α 238A allele/238G allele primers, DNA, then the volume was completed to 20 µL by Deionized sterile water.

PCR reaction was done using a PCR system (9700 thermocycler, Singapore). The mixture was incubated at 95 °C for 10 minutes, followed by 5 cycles of 95 °C for 1 minute, 60 °C for 1 minute, 72 °C for 1 minute, then 25 cycles of 95 °C for 1 minute, 56°C for 1 min, 72 °C for 1 minute, and then a final 10 minutes at 72°C (13). The products were resolved in 1.5% agarose gel, stained with ethidium bromide, and visualized under UV light.

Statistical analysis

Data were presented as means with their standard deviations. The SPSS (version 20.0) and StatDisk (version 13.0) were used for data analysis. T-test, correlation test, and One Way ANOVA were used to compare the results, at a 95% confidence interval, *P value* < 0.05 was considered as significant.

Results

The study was conducted on 100 children with severe falciparum malaria (SM) (mean age 8.63 ± 3.40 years; 61% boys; 49% girls), and 100 children with uncomplicated falciparum malaria (UM) (mean age 8.83 ± 4.20 years; 45% boys; 55% girls) from Gezira State, Sudan. Falciparum malaria-related anemia accounted for 32%, commonly in SM (55%) compared to UM (9%). Severe malaria anemia is most common in SM (3%). Fever was the most clinical finding account for 89% in SM and 81% in UM (Table 1).

TNF-α 238 alleles polymorphism represent (130 [65%] for UM, 137 [68.5%] for SM) for (TNF-α 238A), (70 [35%] for UM, 63 [31.5%] for SM) for (TNF-α 238G) (Figures 2 and 3).

TNF-α 238 GA, AA, and GG account for (58, 36, and 6% respectively) in UM; while (51, 43, and 6% respectively) in SM (Figure 4).

The average of TNF-α levels in severe malaria and UM were (200.98 ± 92.77 and 112.42 ± 35.52 pg /mL respectively) ($P = 0.000$). The average TNF-α levels in anemic patients (196.34 ± 94.11 pg /mL) was higher than in non-anemic patients (122.97 ± 49.45 pg /mL) ($P = 0.000$). The average of TNF-α levels in mild anemia, moderate anemia, and severe anemia was (190.75 ± 102.55, 189.70 ± 80.35 and 299.75 ± 82.27 pg /mL respectively)

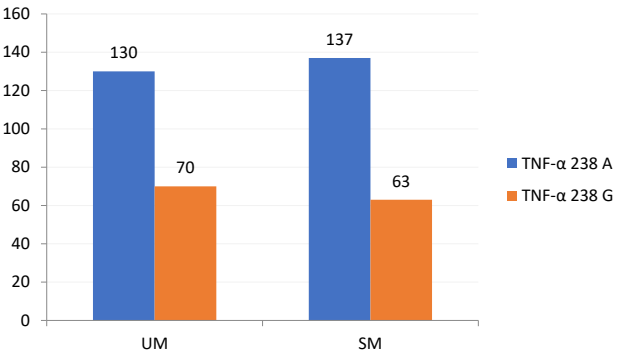

Figure. 2. Frequency of TNF-α 238 Alleles Polymorphism Among UM and SM. UM, Uncomplicated malaria; SM, Severe malaria; TNF-α, Tumor necrosis factor alpha

Table 1. Demographic and clinical characteristics of study participants

Variables	UM (n=100)	SM (n=100)	All Malaria Cases (N=200)
Age (y) (Mean±SD)	8.83±4.20	8.63±3.40	8.43±3.80
Age group (y)			
<5	24 (24%)	19 (19%)	43 (21.5%)
6–10	41 (41%)	47 (47%)	88 (44%)
11–15	29 (29%)	33 (33%)	62 (31%)
>15	6 (6%)	1 (1%)	7 (3.5%)
Gender			
Boys	45 (45%)	61 (61%)	106 (53%)
Girls	55 (55%)	39 (39%)	94 (47%)
Residence			
Rural	70 (70%)	49 (49%)	119 (59.5)
Urban	30 (30%)	51 (51%)	81 (40.5%)
Clinical findings			
Fever	89 (89%)	81 (81%)	170 (85%)
Chills	40 (40%)	27 (27%)	67 (33.5%)
Fatigue	43 (43%)	59 (59%)	102 (51%)
Anemia			
Anemic	55 (55%)	9 (9%)	64 (32%)
Non-anemic	45 (45%)	91 (91%)	136 (68%)
Severity of the anemia			
Mild	23 (23%)	8 (8%)	31 (15.5%)
Moderate	29 (29%)	1 (1%)	30 (15%)
Severe	3 (3%)	-	3 (1.5%)
TNF-α (pg/mL), Mean±SD	112.42±35.52	200.98±92.77	156.70±64.15

UM, Uncomplicated malaria; SM, Severe malaria; TNF-α, Tumor necrosis factor alpha.

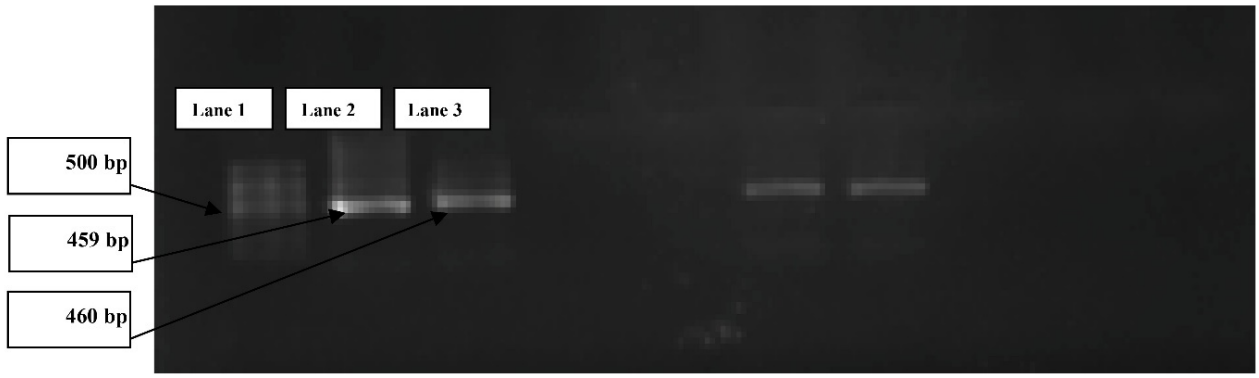

Figure 3. PCR Amplification of TNF-α 238 alleles DNA on 1.5% Agarose Gel Electrophoresis. Lane 1 DNA ladder: MW 500-1500bp. Lane 2 showing band size of (460 bp) for G allele, Lane 3 showing band size of (459 bp) for A allele

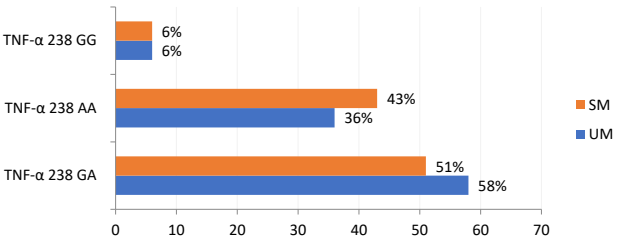

Figure 4. Frequency of TNF-α 238 Genotypes Polymorphism Among UM and SM. UM, Uncomplicated malaria; SM, Severe malaria; TNF-α, Tumor necrosis factor alpha

giving highly significant differences between them ($P=0.000$) and strong significant positive correlation ($r+0.309$; $P=0.000$) (Table 2).

TNF-α 238 GA, AA and GG genotypes account for (58, 36 and 6% respectively) in UM; while (51, 43 and 6% respectively) in UM ($P=0.586$) (Table 3).

TNF-α 238 AA account for 47 (73.4%) in anemic patients (70.9% in SM, 88.9% in UM), giving highly significant association between TNF-α 238 AA and malaria anemia ($P=0.000$); in both SM ($P=0.000$) and

UM ($P=0.015$) (Table 4).

TNF-α 238 AA account for (21 [67.7%], 24 [80%] and 2 [66.7%] respectively) in mild, moderate and severe anemia, giving a highly significant association between TNF-α 238 AA and clinical types of malaria anemia ($P=0.000$), and also in SM ($P=0.000$) (Table 5).

The risk difference (RD) of TNF-α 238 A allele for SM and malaria anemia (7.5 and 24.8% respectively); while The risk ratio (RR) of TNF-α 238 A allele for SM and malaria anemia were (1.10 and 1.42 times respectively). The RD of TNF-α 238 AA for SM and malaria anemia were (7.5 and 53.3% respectively); while The RR of TNF-α 238 AA for SM and malaria anemia were (1.20 and 3.13 times respectively) (Table 6).

Discussion

Falciparum malaria is still a major health problem in Sudan accounts for up to 80% of malaria cases globally (2) and about 87.6% of malaria cases in Sudan (3,21,22). Poor sanitation and the absence of major protection are significantly leading to increased prevalence of the disease. Children suffer more malaria episodes and are more prone to severe malaria compared to adults and accounted for 61% (266 000) of all malaria deaths. In fact, about 285,000 children died before their fifth birthdays in 2016 in Africa According to the World Health Organization (2,5). Therefore malaria remains the largest cause of childhood deaths in Africa (23).

TNF-α is a common proinflammatory cytokine. TNF-α is playing a central role in malaria pathogenicity either in the cure or complication of malaria. Their high level and is associated with severe falciparum malaria and is equivocal.

The current research aimed to light the association between TNF-α levels and TNF-α 238 alleles polymorphism with malaria severity and malaria anemia because thought TNF-α levels and their promoter is one of children predispose factor than others lead to malaria anemia.

Polymorphisms in the TNF-α gene have been associated with increased susceptibility to severe malaria. The TNF-α promoter polymorphism at TNF-α 238 alleles have been associated with differential activity and production of TNF-α in addition associated with severe clinical outcome of malaria (11,16).

The present study was conducted on 200 Sudanese children from Gezira State. Samples were collected from 100 subjects (with mean age 8.63 ± 3.40 years; 61% boys) previously diagnosed as severe *falciparum* malaria (SM) by blood film and WHO criteria (19); 100 subjects

Table 2. Comparison of TNF-α Levels Between Severe and Uncomplicated Falciparum Malaria

Factors	TNF-α (pg /mL) Mean ± SD	*P* Value
Groups		0.000
SM	200.98 ± 92.77	
UM	112.42 ± 35.52	
Anemia		0.000
Anemic	196.34 ± 94.11	
Non-anemic	122.97 ± 49.45	
Clinical anemia		0.000
Mild anemia	190.75 ± 102.55	
Moderate anemia	189.70 ± 80.35	
Severe anemia	299.75 ± 82.27	

UM, Uncomplicated malaria; SM, Severe malaria; TNF-α, Tumor necrosis factor alpha.

Table 3. Association Between TNF-α 238 Genotypes Polymorphism and Malaria Severity

Polymorphism	UM	SM	*P* Value
TNF-α 238 GA	58	51	
TNF-α 238 AA	36	43	0.586
TNF-α 238 GG	6	6	

UM, Uncomplicated malaria; SM, Severe malaria; TNF-α, Tumor necrosis factor alpha.

Table 4. Association Between TNF-α 238 Genotypes Polymorphism & Malaria Anemia

Polymorphism	Malaria Patients = 200			SM = 100			UM = 100		
	Anemic	Non-anemic	*P* Value	Anemic	Non-anemic	*P* Value	Anemic	Non-anemic	*P* Value
238 GA	13	96		12	39		1	57	
238 AA	47	32	0.000	39	4	0.000	8	28	0.015
238 GG	4	8		4	2		0	6	

UM, Uncomplicated malaria; SM, Severe malaria; TNF-α, Tumor necrosis factor alpha.
* *P* value < 0.05 .

Table 5. Association between TNF-α 238 genotypes polymorphism and clinical types of anemia

Polymorphism	Malaria patients = 200				SM = 100			
	Mild Anemia	Moderate Anemia	Severe anemia	*P* value *	Mild anemia	Moderate anemia	Severe anemia	*P* value *
238 GA	7	5	1		6	5	1	
238 AA	21	24	2	0.000	14	23	2	0.000
238 GG	3	1	0		3	1	0	

* *P* value < 0.05

Table 6. Risk Difference (RD) and Risk Ratio (RR) for TNF-α 238 A Allele and AA Genotype in Severe malariaand Malaria Anemia

Factors	Severe malaria	Malaria anemia
RD for TNF-α 238 A allele	3.5%	24.8%
RR for TNF-α 238 A allele	1.10	1.42
RD for TNF-α 238 AA genotype	7.5%	53.3%
RR for TNF-α 238 AA genotype	1.20	3.13

(with mean age 8.83 ± 4.20 years; 45% boys) previously diagnosed as uncomplicated *falciparum* malaria (UM) by blood film or ICT and 100 normal healthy controls. Similar studies were reported from different countries like Nigeria (24), Ethiopia (25), and Ghana (26). In the present study boys more than girls. Similarly, a survey was done in Sudan in 21,988 individuals to show the prevalence of malaria and results showed the infection was higher in males more than females (27).

The classical clinical finding was fever (81% for UM, 89% for SM). A study done by Rathod et al showed that fever account for 97% of falciparum malaria (28).

TNF-α 238A allele was a common allele (66.8%) among Sudanese children with falciparum malaria (35.5% for boys and 31.3% for girls), and the G allele was rare (33.2%) (17.5% for boys and 15.7% for girls) (P value 0.132). While TNF-α 238 GA, AA, and GG account for (54.5, 39.5, and 6% respectively) among Sudanese children with falciparum. TNFA-α 238G was the common allele (0.95), and TNFA-α 238A was rare in Malian children (15), and the allele frequencies were 97.8% and 2.2% for TNF-α 238G and TNF-α 238A in Burkina Faso children (29).

Falciparum malaria-related anemia accounted for 32%, commonly in SM (55%) compared to UM (9%). Mild malaria anemia, moderate malaria anemia and severe malaria anemia were accounts for 31%, 30%, and 3% respectively. A previous study reported malaria-related anemia prevalent among children was 19.8% in Cameron and prevalence of mild, moderate, and severe malaria anemia were 88.1, 5.6, and 5.6% respectively (30). The study done by Rathod et al showed the malaria anemia account for 24.6% of falciparum malaria (28). A similar study done in Sudan showed that malaria anemia account for 21.8% of falciparum malaria (31).

The average of TNF-α levels in anemic (196.34 ± 94.11 pg /mL) was higher than the non-anemic patients (122.97 ± 49.45 pg /mL) giving highly significant differences between them ($P = 0.000$ and $P = 0.004$ respectively). Elevated plasma TNF-α levels promote the development of malaria-related in children (32-34). In contrast, McGuire et al reported no association between TNF levels and malaria anemia (13). The TNF-α overproduction in malaria can contribute to reduced red cell production and anemia through suppression of bone marrow erythropoiesis and dyserythropoiesis. TNF-α has been shown to suppress erythropoiesis through inhibition of BFU-E and CFU-E through decreasing their responsiveness to erythropoietin (12,13,18,35,36). Also, the suppressive effect of macrophages from patients on human BFU-E and CFU-E was shown to be mediated by TNF-α (18). Furthermore, TNF-α synergizes with hemozoin and NO in the inhibition of erythropoiesis (34, 37). On the other hand, Elevated TNF-α level and GM-CSF synergistically increase FcγR and CR expression on human neutrophils and monocytes thereby stimulated opsonin-dependent phagocytosis and thereby enhanced clearance of parasitized erythrocytes but the prolonged response was seen to contribute to adverse disease and thus was associated with severe disease syndromes (32,38). Also, TNF-α overproduction in malaria may result from the upregulation of the expression of endothelial adhesion molecules such as ICAM-1 or other adhesion molecules, leading to enhanced sequestration of parasitized red cells and anemia through macrophage activation (18,39). More ever reduced prostaglandin E2 production by hemozoin is reported to lead to overproduction of TNF-α and anemia (33,40). In addition, TNF-α contributes to the anemia of chronic disease by the suppression of erythropoiesis and reduced erythroblast iron incorporation (18,41).

The average of TNF-α levels in mild, moderate and severe anemia were (190.75 ± 102.55, 189.70 ± 80.35 and 299.75 ± 82.27 pg /mL respectively) giving highly significant differences between them ($P = 0.000$) and strong significant positive correlation (r + 0.309; $P = 0.000$), and significant negative correlation with Hb (r – 0.419; $P = 0.000$). Similar study showed mild, moderate and severe anemia were (108.9, 132.2 and 193.9 pg /mL respectively), giving highly significant differences between them and strong significant positive correlation with anemia severity and negative correlation with Hb (42). Direct associations between severe malarial anemia was found for higher TNF-α concentration in children in Zambia (32), Kenya (43), Ghana (44) and Pakistani (42). In contrast other study have found no association between or high TNF-α and malarial anemia (45). In *P. falciparum* infected children, the TNF level was negative correlated with Hb levels (38, 46). Continuous TNF-α overproduction increase suppression of bone marrow erythropoiesis and dyserythropoiesis and accelerated destruction of infected red blood cells result in enhancing severity of malaria anemia (18,32,35,36,38). In *P. falciparum* infected children, the TNF-α level was negatively correlated with Hb levels (38,46,47).

TNF-α 238 A account for 53.5 (83.6%) from 64 anemic patients, giving highly significant association between TNF-α 238 A alleles (especially TNF-α 238 AA genotype [73.4%]) with malaria anemia compared to G alleles ($P = 0.000$), in both SM (P value 0.000) and UM ($P = 0.015$) within TNF-α 238 A allele to be associated with susceptibility to 3.13 fold risk for developing anemia. Furthermore, TNF-α 238 AA represent (67.7%, 80% and

66.7% respectively) in mild, moderate and severe anemia; giving highly significant association between TNF-α 238 A alleles and clinical types of malaria anemia ($P=0.000$), and also in SM ($P=0.000$). This finding consistence with study done in Gambia that found TNF-α 238 A allele to be associated with susceptibility to malarial anemia especially severe malaria anemia with a 2.5 fold risk of developing SMA ($P<0.001$) (13). May et al reported TNF-α 238 alleles were associated with severe malaria anemia (48). In contrast, study done in Mali reported lack of association between TNF-α 238 alleles and severe malaria anemia (15). This suggest that the location of the TNF-α 238 A allele in the TNF-α promoter region to be associated with susceptibility to influence constitutive TNF-α production directly compared to G allele in malaria anemia. SNPs at many positions as 238 in the proximal enhancer of the TNF gene exhibit differential associations to malaria and TNF production in different populations (49-52).

The confounders of other baseline factors were not addressed here; we suggest a more details on baseline characteristics like socioeconomic status, nutritional status and co-infection could help rule out potential confounders.

Conclusion

The significance of TNF-α level and TNF-α 238 A allele in children with severe falciparum anemia will assist clinicians in diagnosing and better managing severe malaria cases.

Acknowledgements

The authors would like to thank all participants recruited for the study. We also thank the medical staff of the Wad Medani Pediatric Hospital for their help during the study.

Authors' Contribution

Conceptualization: Khalid Abdelsamea Mohamedahmed, Adam Dawoud Abakar, Yousef Gharedaghi, Salah Eldin G. Elzaki, Yagoob Garedaghi, Roa Osman Elsumani Koko.
Data curation: Adam Dawoud Abakar, Yousef Gharedaghi, Salah Eldin G. Elzaki, Roa Osman Elsumani Koko.
Formal analysis: Mohamed Youisf Mohamed Elshiekh, Adam Dawoud Abakar, Yousef Gharedaghi, Roa Osman Elsumani Koko.
Investigation: Khalid Abdelsamea Mohamedahmed, Mohamed Youisf Mohamed Elshiekh, Adam Dawoud Abakar, Yagoob Garedaghi, Roa Osman Elsumani Koko.
Methodology: Khalid Abdelsamea Mohamedahmed, Bakri Yousif Mohammed Nour, Mohamed Youisf Mohamed Elshiekh, Adam Dawoud Abakar.
Project administration: Khalid Abdelsamea Mohamedahmed, Mohamed Youisf Mohamed Elshiekh, Adam Dawoud Abakar.
Resources: Khalid Abdelsamea Mohamedahmed, Yousef Gharedaghi, Yagoob Garedaghi.
Software: Bakri Yousif Mohammed Nour, Mohamed Youisf Mohamed Elshiekh, Adam Dawoud Abakar, Yousef Gharedaghi.
Supervision: Khalid Abdelsamea Mohamedahmed, Yousef Gharedaghi, Yagoob Garedaghi.
Validation: Khalid Abdelsamea Mohamedahmed, Bakri Yousif Mohammed Nour, Mohamed Youisf Mohamed Elshiekh.
Visualization: Khalid Abdelsamea Mohamedahmed, Yousef Gharedaghi, Yagoob Garedaghi.
Writing–original draft: Khalid Abdelsamea Mohamedahmed.
Writing–review & editing: Khalid Abdelsamea Mohamedahmed, Yagoob Garedaghi.

Competing Interests

None.

Data Availability Statement

Data are presented within the manuscript and can be provided by the corresponding author upon reasonable request.

Ethical Approval

Ethical approval was obtained from the both Researches and Ethics Committees (REC) of Ministry of Health, Gezira State (No: 4-11-2017). Informed consent was written from each participant.

Funding

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

References

1. Recker M, Bull PC, Buckee CO. Recent advances in the molecular epidemiology of clinical malaria. F1000Res. 2018;7:F1000 Faculty Rev-1159. doi: 10.12688/f1000research.14991.1.
2. World Health Organization (WHO). World Malaria Report 2018. 11th ed. Geneva: WHO; 2018.
3. Mohamedahmed KA, Mustafa RE, Abakar AD, Nour BY. Evaluation of Neutrophil Lymphocyte Ratio (NLR) in Sudanese children with falciparum malaria. Int J Acad Health Med Res. 2019;3(5):1-6.
4. Mohamedahmed KA, Nour BY, Abakar AD, Babker AM. Diagnostic and prognostic value of thrombocytopenia severity in Sudanese children with falciparum malaria. World J Adv Res Rev. 2020;6(3):197-204. doi: 10.30574/wjarr.2020.6.3.0196.
5. Mohamedahmed KA, Abakar AD. Severe falciparum malaria: an overview. Int J Med Parasitol Epidemiol Sci. 2020;1(4):105-6. doi 10.34172/ijmpes.2020.28.
6. Mawson AR. The pathogenesis of malaria: a new perspective. Pathog Glob Health. 2013;107(3):122-9. doi: 10.1179/2047773213y.0000000084.
7. Antwi-Baffour S, Kyeremeh R, Buabeng D, Adjei JK, Aryeh C, Kpentey G, et al. Correlation of malaria parasitaemia with peripheral blood monocyte to lymphocyte ratio as indicator of susceptibility to severe malaria in Ghanaian children. Malar J. 2018;17(1):419. doi: 10.1186/s12936-018-2569-x.
8. Mohamedahmed KA, Ahmed ZA, Nour BY, Abakar AD, Babker AM. Impact of sever *Plasmodium falciparum* infection on platelets parameters among Sudanese children living in Al-Jazira state. Int J Clin Biomed Res. 2020;6(2):5-9. doi: 10.31878/ijcbr.2020.62.02.
9. Urban BC, Willcox N, Roberts DJ. A role for CD36 in the regulation of dendritic cell function. Proc Natl Acad Sci U S A. 2001;98(15):8750-5. doi: 10.1073/pnas.151028698.
10. Mohamedahmed KA, Abakar AD, Omer M, Mukhtar MM, Nour BY. The role of TNF-α levels as predictive diagnostic biomarker among children with severe falciparum malaria in endemic area in Sudan. Int J Acad Health Med Res. 2019;3(7):1-6.
11. Flori L, Delahaye NF, Iraqi FA, Hernandez-Valladares M, Fumoux F, Rihet P. TNF as a malaria candidate gene: polymorphism-screening and family-based association analysis of mild malaria attack and parasitemia in Burkina Faso. Genes Immun. 2005;6(6):472-80. doi: 10.1038/sj.gene.6364231.

12. Mohamedahmed KA. Association between elevated TNF-α levels and severe malaria. Galen Med J. 2023;12:e2927. doi: 10.31661/gmj.v12i0.2927.
13. McGuire W, Knight JC, Hill AV, Allsopp CE, Greenwood BM, Kwiatkowski D. Severe malarial anemia and cerebral malaria are associated with different tumor necrosis factor promoter alleles. J Infect Dis. 1999;179(1):287-90. doi: 10.1086/314533.
14. Aidoo M, McElroy PD, Kolczak MS, Terlouw DJ, ter Kuile FO, Nahlen B, et al. Tumor necrosis factor-alpha promoter variant 2 (TNF2) is associated with pre-term delivery, infant mortality, and malaria morbidity in western Kenya: Asembo Bay Cohort Project IX. Genet Epidemiol. 2001;21(3):201-11. doi: 10.1002/gepi.1029.
15. Cabantous S, Doumbo O, Ranque S, Poudiougou B, Traore A, Hou X, et al. Alleles 308A and 238A in the tumor necrosis factor alpha gene promoter do not increase the risk of severe malaria in children with *Plasmodium falciparum* infection in Mali. Infect Immun. 2006;74(12):7040-2. doi: 10.1128/iai.01581-05.
16. Ubalee R, Suzuki F, Kikuchi M, Tasanor O, Wattanagoon Y, Ruangweerayut R, et al. Strong association of a tumor necrosis factor-alpha promoter allele with cerebral malaria in Myanmar. Tissue Antigens. 2001;58(6):407-10. doi: 10.1034/j.1399-0039.2001.580610.x.
17. McGuire W, Hill AV, Allsopp CE, Greenwood BM, Kwiatkowski D. Variation in the TNF-alpha promoter region associated with susceptibility to cerebral malaria. Nature. 1994;371(6497):508-10. doi: 10.1038/371508a0.
18. Abdalla SH, Pasvol G. Malaria: A Hematological Perspective (Tropical Medicine: Science and Practice. London: Imperial College Press; 2004.
19. World Health Organization (WHO). Guidelines for the Treatment of Malaria. 3rd ed. Geneva: WHO; 2015.
20. Bain BJ, Bates I, Laffan MA, S. Lewis M. Dacie and Lewis Practical Haematology. 11th ed. Elsevier Ltd; 2011.
21. Mohammed ZO, Gaberallah K, Mohammed MS, Mohamedahmed KA. Evaluation of coagulation profiles (PT, INR, and APTT) among Sudanese patients with falciparum malaria infection. Int J Acad Health Med Res. 2020;4(11):15-21.
22. Bilal JA, Gasim GI, Abdien MT, Elmardi KA, Malik EM, Adam I. Poor adherence to the malaria management protocol among health workers attending under-five year old febrile children at Omdurman hospital, Sudan. Malar J. 2015;14:34. doi:10.1186/s12936-015-0575-9.
23. Roberts D, Matthews G. Risk factors of malaria in children under the age of five years old in Uganda. Malar J. 2016;15:246. doi: 10.1186/s12936-016-1290-x.
24. Madukaku CU, Chimezie OM, Chima NG, Hope O, Simplicius DIN. Assessment of the haematological profile of children with malaria parasitaemia treated with three different artemisinin-based combination therapies. Asian Pac J Trop Dis. 2015;5(6):448-53. doi: 10.1016/s2222-1808(15)60813-1.
25. Birhanu M, Asres Y, Adissu W, Yemane T, Zemene E, Gedefaw L. Hematological parameters and hemozoin-containing leukocytes and their association with disease severity among malaria infected children: a cross-sectional study at Pawe General Hospital, Northwest Ethiopia. Interdiscip Perspect Infect Dis. 2017;2017:8965729. doi: 10.1155/2017/8965729.
26. Frimpong A, Kusi KA, Tornyigah B, Ofori MF, Ndifon W. Characterization of T cell activation and regulation in children with asymptomatic *Plasmodium falciparum* infection. Malar J. 2018;17(1):263. doi: 10.1186/s12936-018-2410-6.
27. Bhatt S, Weiss DJ, Cameron E, Bisanzio D, Mappin B, Dalrymple U, *et al*. The effect of malaria control on Plasmodium falciparum in Africa between 2000 and 2015. Nature. 2015;526:207-211. doi:10.1038/nature15535
28. Rathod CC, Deshpande SV, Rana HM, Godbole VY, Patel A, Patel V, et al. *Plasmodium falciparum* versus *Plasmodium vivax*: which is a lesser evil? Natl J Community Med. 2012;3(3):541-7.
29. Flori L, Sawadogo S, Esnault C, Delahaye NF, Fumoux F, Rihet P. Linkage of mild malaria to the major histocompatibility complex in families living in Burkina Faso. Hum Mol Genet. 2003;12(4):375-8. doi: 10.1093/hmg/ddg033.
30. Sumbele IU, Kimbi HK, Ndamukong-Nyanga JL, Nweboh M, Anchang-Kimbi JK, Lum E, et al. Malarial anaemia and anaemia severity in apparently healthy primary school children in urban and rural settings in the Mount Cameroon area: cross sectional survey. PLoS One. 2015;10(4):e0123549. doi: 10.1371/journal.pone.0123549.
31. Ahamed AM, Hobiel HA, Modawe GA, Elsammani MS. Hematological changes in Sudanese patients with falciparum malaria attending Elnihoud Teaching Hospital. Sudan J Med Sci. 2019;14(1):24-30. doi: 10.18502/sjms.v14i1.4378.
32. Thuma PE, van Dijk J, Bucala R, Debebe Z, Nekhai S, Kuddo T, et al. Distinct clinical and immunologic profiles in severe malarial anemia and cerebral malaria in Zambia. J Infect Dis. 2011;203(2):211-9. doi: 10.1093/infdis/jiq041.
33. Daffa Alla N, Sukkar MY. IFN-γ, TNF-α and IL-10 responses in children infected with malaria parasite. Khartoum Med J. 2015;8(3):1143-52.
34. Mandala WL, Msefula CL, Gondwe EN, Drayson MT, Molyneux ME, MacLennan CA. Cytokine profiles in Malawian children presenting with uncomplicated malaria, severe malarial anemia, and cerebral malaria. Clin Vaccine Immunol. 2017;24(4):e00533-16. doi: 10.1128/cvi.00533-16.
35. Ekvall H. Malaria and anemia. Curr Opin Hematol. 2003;10(2):108-14. doi: 10.1097/00062752-200303000-00002.
36. Robson KJ, Weatherall DJ. Malarial anemia and STAT6. Haematologica. 2009;94(2):157-9. doi: 10.3324/haematol.2008.002311.
37. Awandare GA, Kempaiah P, Ochiel DO, Piazza P, Keller CC, Perkins DJ. Mechanisms of erythropoiesis inhibition by malarial pigment and malaria-induced proinflammatory mediators in an in vitro model. Am J Hematol. 2011;86(2):155-62. doi: 10.1002/ajh.21933.
38. Deroost K, Pham TT, Opdenakker G, Van den Steen PE. The immunological balance between host and parasite in malaria. FEMS Microbiol Rev. 2016;40(2):208-57. doi: 10.1093/femsre/fuv046.
39. Lamikanra AA, Merryweather-Clarke AT, Tipping AJ, Roberts DJ. Distinct mechanisms of inadequate erythropoiesis induced by tumor necrosis factor alpha or malarial pigment. PLoS One. 2015;10(3):e0119836. doi: 10.1371/journal.pone.0119836.
40. Keller CC, Davenport GC, Dickman KR, Hittner JB, Kaplan SS, Weinberg JB, et al. Suppression of prostaglandin E2 by malaria parasite products and antipyretics promotes overproduction of tumor necrosis factor-alpha: association with the pathogenesis of childhood malarial anemia. J Infect Dis. 2006;193(10):1384-93. doi: 10.1086/503047.
41. Spottiswoode N, Duffy PE, Drakesmith H. Iron, anemia and hepcidin in malaria. Front Pharmacol. 2014;5:125. doi: 10.3389/fphar.2014.00125.
42. Gandapur AS, Malik SA. Tumor necrosis factor in falciparum malaria. Ann Saudi Med. 1996;16(6):609-14. doi: 10.5144/0256-4947.1996.609.
43. Othoro C, Lal AA, Nahlen B, Koech D, Orago AS, Udhayakumar V. A low interleukin-10 tumor necrosis factor-alpha ratio is associated with malaria anemia in children residing in a holoendemic malaria region in western Kenya. J Infect Dis. 1999;179(1):279-82. doi: 10.1086/314548.

44. Kurtzhals JA, Adabayeri V, Goka BQ, Akanmori BD, Oliver-Commey JO, Nkrumah FK, et al. Low plasma concentrations of interleukin 10 in severe malarial anaemia compared with cerebral and uncomplicated malaria. Lancet. 1998;351(9118):1768-72. doi: 10.1016/s0140-6736(97)09439-7.
45. Helleberg M, Goka BQ, Akanmori BD, Obeng-Adjei G, Rodriques O, Kurtzhals JA. Bone marrow suppression and severe anaemia associated with persistent *Plasmodium falciparum* infection in African children with microscopically undetectable parasitaemia. Malar J. 2005;4:56. doi: 10.1186/1475-2875-4-56.
46. Ogonda LA, Orago AS, Otieno MF, Adhiambo C, Otieno W, Stoute JA. The levels of CD16/Fc gamma receptor IIIA on CD14+CD16+monocytes are higher in children with severe *Plasmodium falciparum* anemia than in children with cerebral or uncomplicated malaria. Infect Immun. 2010;78(5):2173-81. doi: 10.1128/iai.01078-09.
47. Nussenblatt V, Mukasa G, Metzger A, Ndeezi G, Garrett E, Semba RD. Anemia and interleukin-10, tumor necrosis factor alpha, and erythropoietin levels among children with acute, uncomplicated *Plasmodium falciparum* malaria. Clin Diagn Lab Immunol. 2001;8(6):1164-70. doi: 10.1128/cdli.8.6.1164-1170.2001.
48. May J, Lell B, Luty AJ, Meyer CG, Kremsner PG. Plasma interleukin-10: tumor necrosis factor (TNF)-alpha ratio is associated with TNF promoter variants and predicts malarial complications. J Infect Dis. 2000;182(5):1570-3. doi: 10.1086/315857.
49. Sinha S, Mishra SK, Sharma S, Patibandla PK, Mallick PK, Sharma SK, et al. Polymorphisms of TNF-enhancer and gene for FcgammaRIIa correlate with the severity of falciparum malaria in the ethnically diverse Indian population. Malar J. 2008;7:13. doi: 10.1186/1475-2875-7-13.
50. Garedaghi Y, Bahavarnia SR. Repairing effect of *Allium cepa* on testis degeneration caused by Toxoplasma gondii in the rat. Int J Womens Health Reprod Sci. 2014;2(2):81-9.
51. Garedaghi Y. Seroprevalence of *Neospora caninum* in stray dogs of Tabriz, Iran. J Anim Vet Adv. 2012:11(6):723-726.
52. Rahman HU, Khatoon N, Arshad S, Masood Z, Ahmad B, Khan W, et al. Prevalence of intestinal nematodes infection in school children of urban areas of district Lower Dir, Pakistan. Braz J Biol. 2022;82:e244158. doi: 10.1590/1519-6984.244158.