Original Research Article

Association of Haptoglobin Gene polymorphism with Uncomplicated Malaria Cases in Elfasher- Northern Darfur State

Abstract

Background: Malaria is an acute febrile illness caused by *Plasmodium* parasites. Malaria was remain one of the major problems in Sudan, recently the emergence of malaria in Northern Darfur lead to the outbreak in the 2018 and 2019 was lead to high morbidity and mortality , addressing the host parasites interaction may be one the reason.
Haptoglobin is an acute phase protein capable of binding hemoglobin, thus preventing iron loss and renal damage, also acts as an antioxidant, has antibacterial activity and plays a role in modulating many aspects of the acute phase response.
Methodology: To determine polymorphism in Haptoglobin genes in malaria patients, using polymerase chain reaction (PCR) in Elfasher city Northern Darfur State, durning the transmission season of August 2020 to December 2021. A total of 142 individuals were included, 26.8% males and 73.2%females.Data variables were reported using chi square test. Comparisons of continuous variables using the one-way analysis of variance ANOVA for parametric data and Kruskal Wallis test for non-parametric data. An alpha value of < 0.05 denoted a statistically significant difference in all statistical comparisms.
Results: Association of Hp genotypes and the risk of uncomplicated malaria were analyzed by age and gender. Hp1-1 genotype was most frequency (44.4%) compared to other genotypes. The Hp1-1, Hp2-1 and Hp2-2 genotypes were found in 88.9%%, 100% and 71.1% of female patients, respectively. None of age and gender factors revealed statistically significant association ($P = 0.05$).
The Hp2-2 genotype was higher with 89.2% on malaria patients with fever compared to other genotypes. It is possible that the Hp2-2 genotype protects against a range of malaria symptoms, but we did not find a significant association between Hp genotypes and malaria clinical symptoms ($P = 0.05$)
Conclusion: This study showed the Hp 1–1 and Hp 2–1 genotypes each occur in nearly 4 in 10 children and the Hp 2–2 genotype occurs in 2 of 10 children. No association with incidence of uncomplicated malaria was found. Additional studies of influence of Haptoglobin genotypes on *P. falciparum* malaria severity are needed to understand the role of these genotypes in malarial protection

Keywords: Haptoglobin, Malaria,CBC, Sudan,

1. INTRODUCTION

Malaria is an acute febrile illness caused by *Plasmodium* parasites, which are spread to people through the bites of infected female *Anopheles* mosquitoes. There are 5 parasite species that cause malaria in humans, and 2 of these species – *P. falciparum* and *P. vivax* – pose the greatest threat. *P. falciparum* is the deadliest malaria parasite and the most prevalent on the African continent. *P. vivax* is the dominant malaria parasite in most countries outside of sub-Saharan Africa. Which can be asymptomatic or develop into mild symptoms including fever, and a minority of infections progress to severe complications that might result in death [1]

In 2020, nearly half of the world's population was at risk of malaria. High risk groups of contracting malaria and developing severe disease are infants, children under 5 years of age, pregnant women and patients with HIV/AIDS, as well as people with low immunity moving to areas with intense malaria transmission such as migrant workers, mobile p In 2020, nearly half of the world's population was at risk of malaria. High risk groups of contracting malaria and developing severe disease are infants, children under 5 years of age, pregnant women and patients with HIV/AIDS, as well as people with low immunity moving to areas with intense malaria transmission such as migrant workers, mobile populations and travelers [2].

Haptoglobin is an acute phase protein capable of binding haemoglobin, thus preventing iron loss and renal damage. Haptoglobin also acts as an antioxidant, has antibacterial activity and plays a role in modulating many aspects of the acute phase response. There are 3 major Haptoglobin phenotypes--Hp (1-1), Hp (2-1) and Hp (2-2). Possession of a particular phenotype has been associated with a variety of common disorders (e.g. cardiovascular disease, autoimmune disorders, malignancy), a fact which can only be explained by the idea that possession of a particular phenotype offers some protection against the development of these disorders. Knowledge of phenotype could therefore aid in the prognosis of disease and allow treatment to be better tailored to suit an individual' needs [3].

Haptoglobin irreversibly binds oxygenated cell-free hemoglobin, thus preventing the accumulation of free radicals and resultant oxidative tissue damage. [4] The haptoglobin-hemoglobin complex then is removed from circulation by binding the CD163 receptor on monocytes and macrophages. [5].

Materials and methods

This was across sectional study conducted in the different hospitals and Health centers in Elfasher city of Northern Darfur State during the transmission July to November 2021

Figure 1 Sudan map showing location of Northern Darfur State

The ethical consideration was obtained from state Ministry of Health ethical committee, Northern Darfur.

Blood sample was obtained from 142 patient positive for falciparum malaria. in K_2-EDTA and deliver to the haematology laboratory to perform the full blood count using an automated particle cell counter -Sysmex.

Direct microscopic examination:

Thin films were fixed with methanol, and both thin and thick films were stained with 10% Giemsa stain for 15 minutes. All dried slides placed in slides boxes and were examined by laboratory technologist at the health center laboratory in. The presence of malaria parasites on thick blood smear and the identification of *Plasmodium* species from smear was done, through oil immersed objective (100×), at 1000× magnification. The thick smear was used to determine whether the malaria parasites were present or absent and thin smear was used to identify the type of *Plasmodium* species. During the microscopic examination, a slide was regarded as negative after 200 fields had been examined without finding of *Plasmodium* parasite by two laboratory technologists. To assure quality of the microscopic examinations, all positive and 10% of the negative slides were reexamined by a third reader to remove discrepant result.

[6]

Rapid diagnostic tests for malaria (RDT)

Malaria rapid diagnostic tests emerged in the early 1990s into largely unregulated markets, and uncertain field performance was a major concern for the acceptance of tests for malaria case management. RDT was performed in lab from 15 micrometer EDTA blood sample with 2 drops from buffer and the result was recorded within 10 mins. [7-8]

Haptoglobin Genotyping

The Haptoglobin genotype was determined by PCR as described previously [9]with few modifications. Briefly in total volume of 25 µL reactions contained 5µl genomic DNA, 5 µl of ready master mix (Solis BioDyne, Estonia (Qiagen, Hilden, Germany), and 0.25 µmol/L of each primer (A/B). Oligonucleotide primers A and B were used for amplification of a 1757□bp Hp 1 allele□specific sequence and a 3481□bp Hp 2 allele□specific sequence. Primers were purchased from Macrogen, Koria. The amplification reactions were conducted on a DNA Engine Cycler (BioRad) under the following conditions: initial Denaturation for 3 minutes 94°C; 94°C 30 seconds, 57°C primers A/B for 30 seconds, 72°C for 2 minutes, 35 cycles; and final extension for 10 minutes at 72°C. After amplification 10 µL PCR product of A/B primers were separated on a 1% agarose in 1X TBE buffer.

Results

Of the142 study subjects, their average age was 26.9 ranging from 10 months to 81 years old. The majority of the patient groups were more than 25 years old (58.5%) compared to less than 25 years old (41.5%). The gender distribution was (26.8%) males and (73.2%) females. The level of hemoglobin were in normal range in malaria patients (mean=10.05) and mean platelet was (220,119.). According to parasitemia most of the patients (40.8%) was categorized as intermediate. The characteristics of the study group are summarized in Table 1.

Table 1.Baseline characteristics among patients with malaria infection

Characteristics		**Patient N=142**
Age group N (%)	**<5**	8(5.6)
	5-14	25(17.6)
	15-24	26(18.3)
	25-34	48(33.8)
	35-44	18(12.7)
	≥45	17(12.0)
Gender N (%)	**Male**	38(26.8)
	Female	104(73.2)
Haemoglobin (g/dL) mean(range)		10.05(4-14)
Platelet(µL) mean(range)		2201,197 (50,000-920,000)
Parasite counts N (%)	**Low(+)**	21(14.8)
	Intermediate(++)	58(40.8)
	Slightly high(+++)	52(36.6)
	High(++++)	11(7.7)

N: number of study subjects

The observed Haptoglobin genotype showed Hp 2-2 (90.1%) more frequent than others Hp genotype. The overall allele frequency for the Hp2 allele was (91.9%), while Hp1 allele occurred at an allele frequency of (8.1%) which was in accordance with the Hardy-Weinberg equilibrium. The distribution of the Hp genotypes in the study is presented in Table 2 & figure 1.

Table 2. Distribution of Haptoglobin genotypes in the study group

Hp Genotype	Patients N (%)
Hp1-1	9(6.4)
Hp2-1	5(3.5)
Hp2-2	128(90.1)

Allele Hp1	23(8.1)
Allele Hp2	261(91.9)

The most frequency of parasite count according to age group was as follows: less than 5 years of ages, 62.5% categorized as intermediate; 5-14 years of ages, 48.0% as intermediate; 15-24 years of ages, 42.3% as slightly high; 25-34 years of ages, 43.8% as slightly high; 35-44 years of ages, 38.9% as slightly high; more than 44 years of ages, 52.9% expressed as intermediate. There was no significant association between age group and parasite count (P-value>0.05*)* figure 2.

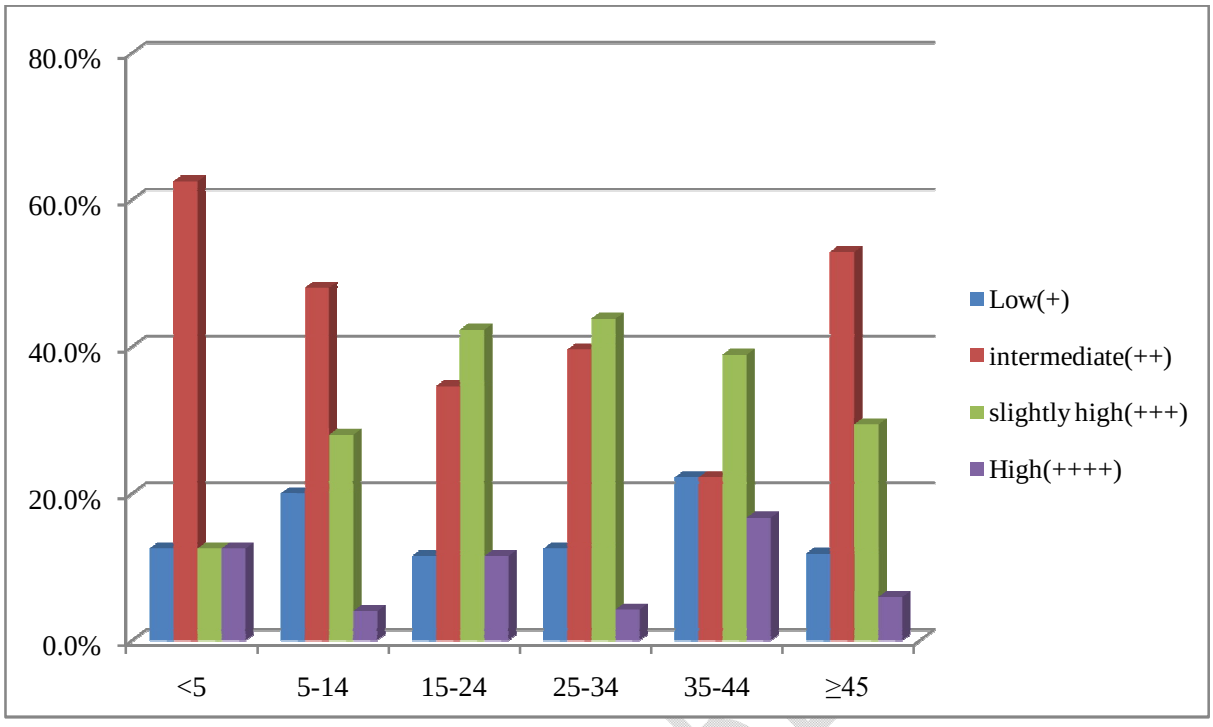

Figure 2. The frequency of parasite count by age group among malaria patients.

Table3. Characteristics of study population, by Haptoglobin genotype

Characteristics	Hp1-1 n=9	Hp2-1 n=5	Hp2-2 n=128	chix^2, p-value
Age group N(%)				
<5	1(11.1)	1(20.0)	6(4.7)	10.55, 0.393
5-14	2(22.2)	0	23(18.0)	

15-24	1(11.1)	0	25(19.5)	
25-34	4(44.4)	2(40.0)	42(32.8)	
35-44	0	0	18(14.1)	
≥45	1(11.1)	2(40.0)	14(10.9)	
Gender N(%)				
Male	1(11.1)	0	37(28.9)	3.25, 0.197
Female	8(88.9)	5(100)	91(71.1)	

P-value>0.05 not statistical significantly

The association of Hp genotypes and the risk of uncomplicated malaria were then analyzed by age and gender. Among age 25-34 years, Hp1-1 genotype was most frequency (44.4%) compared to other genotypes. The Hp1-1, Hp2-1 and Hp2-2 genotypes were found in 88.9%%, 100% and 71.1% of female patients, respectively. None of age and gender factors revealed statistically significant association (P-value>0.05) table 3.

Distribution of Hp genotypes by age group among malaria patients

Of these children group, 33.3% carried the Hp1-1 genotype, 20.0% and 22.7% carried Hp2-1 and Hp2-2 genotypes, respectively. In this figure, the majority of malaria patients were higher in adults with Hp2-1 (80.0%), compared with adults with other genotypes Figure 3.

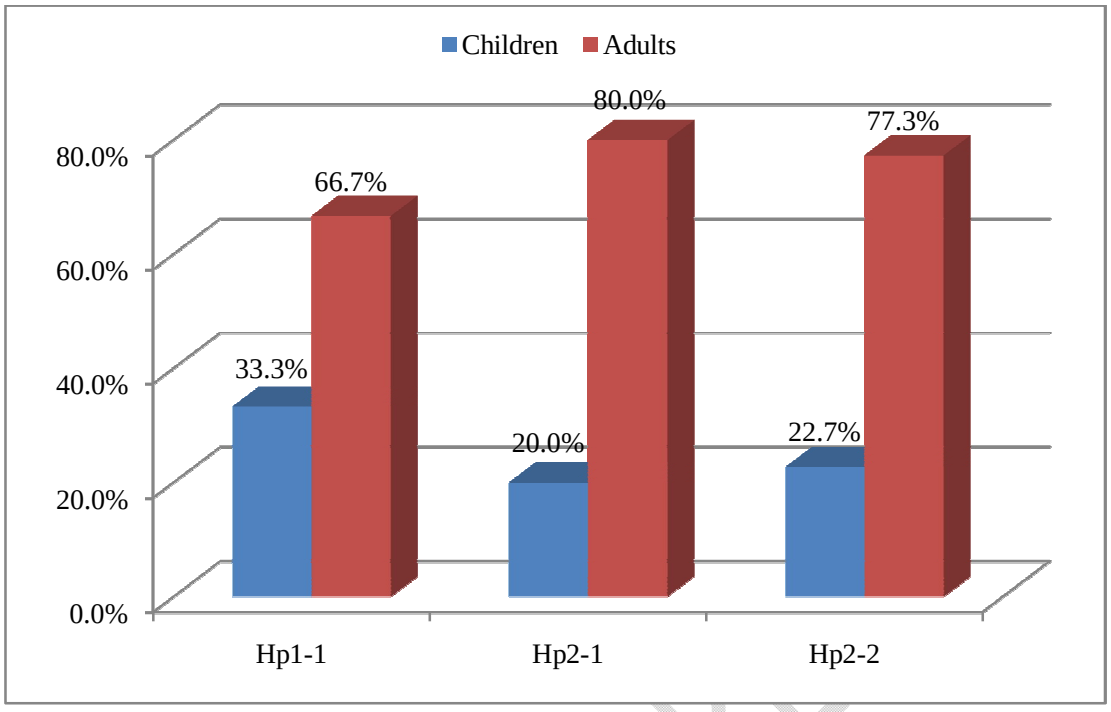

Figure3. Distribution of Hp genotypes by age group among malaria patients

Malaria was studied in relation to Haptoglobin genotypes by level of parasitemia. The results showed that (41.4%) from Hp2-2 were expressed as intermediate while 40.0% and 33.3% were intermediate malaria parasitemia from Hp2-1 and Hp1-1, respectively, but this association was not statistically significant (P-value > 0.05) table 4.

Table 4. Comparison of density in relation to Haptoglobin genotype

Parasitemia	**Hp1-1 N(%)**	**Hp2-1 N(%)**	**Hp2-2 N(%)**	**chix^2, p-value**
Low(+)	3(33.3)	1(20.0)	17(13.3)	4.69, 0.585
Intermediate(++)	3(33.3)	2(40.0)	53(41.4)	
Slightly high(+++)	2(22.2)	1(20.0)	49(38.3)	
High(++++)	1(11.1)	1(20.0)	9(7.0)	

P-value>0.05 not statistical significantly

In this study, the three main Hp genotypes among patients were evaluated against Hb and platelet. The mean Hb level (9.6) was the same in Hp 1-1 andHp2-1, and (10.1) in patients carrying Hp2-2 genotype. For mean platelet level, the Hp1-1 was observed as highest mean (240000) compared to others Hp genotype. All these different were not statistically significant (P-value > 0.05) Table 5.

Table 5. Comparison of hemoglobin and platelet in relation to Haptoglobin genotype

Parameter	**Hp1-1**	**Hp2-1**	**Hp2-2**	**F-test, p-value**
Haemoglobin (g/dL mean(range)	9.56(6-11)	9.60(7-11)	10.1(4-14)	0.82, 0.443
Platelet(µL) mean(range)	240000.0(53000-580000)	190800.0(167000-231000)	219867.19(50000-920000)	0.24, 0.885

P-value>0.05 not statistical significantly

The finding of the study showed the relation between Hp genotypes and parasites count according to age. Children with Hp1-1 genotype were have low parasitemia (66.7%) and Hp2-2 were have intermediate parasitemia(51.7%). At slightly high parasitemia, the adults with Hp2-2 genotype were higher than adults with Hp1-1 or Hp2-1 genotype. No association was found between Hp genotypes and parasite count (P-value>0.05) table 6.

Table 6. Distribution of parasite count by Hp genotypes among children and adults

Parasitemia	**Children N(%)**			**Adults N(%)**		
	Hp1-1	**Hp2-1**	**Hp2-2**	**Hp1-1**	**Hp2-1**	**Hp2-2**
Low(+)	2(66.7)	0	4(13.8)	1(16.7)	1(25.0)	13(13.1)
Intermedite(++)	1(33.3)	1(100)	15(51.7)	2(33.3)	1(25.0)	38(38.4)

Slightly high(+++)	0	0	8(27.6)	2(33.3)	1(25.0)	41(41.4)
High(++++)	0	0	2(6.9)	1(16.7)	1(25.0)	7(7.1)
chix^2, p-value	6.39, 0.380			3.02, 0.806		

P-value>0.05 not statistical significantly

This study also examined the effect of Hp genotypes on malaria parasitemia according to gender. Only one male with Hp1-1 genotype had low parasite count; whereas (48.7%) with Hp2-2 genotype had intermediate parasitemia. Thirty seven (40.7%) female patients with Hp2-2 had slightly high parasitemia. Parasite count among gender did not differ between the Hp genotypes (P-value>0.05) table 7.

Table 7. Distribution of parasite density by Hp genotypes with gender

Parasitemia	**Male N(%)**			**Female N(%)**		
	Hp1-1	**Hp2-1**	**Hp2-2**	**Hp1-1**	**Hp2-1**	**Hp2-2**
Low(+)	1(100)	-	6(16.2)	2(25.0)	1(20.0)	11(12.0)
Intermedite(++)	0	-	18(48.7)	3(37.5)	2(40.0)	35(38.5)
Slightly high(+++)	0	-	12(32.4)	2(25.0)	1(20.0)	37(40.7)
High(++++)	0	-	1(2.7)	1(12.5)	1(20.0)	8(8.8)
chix^2, p-value	4.55, 0.208			2.71, 0.844		

P-value>0.05 not statistical significantly

We found that the Hp2-2 genotype was higher with (89.2%) on malaria patients with fever symptom compared to other genotypes. The same can be said to other symptoms. It is possible that the Hp2-2 genotype protects against a range of malaria symptoms, but we did not find a significant association between Hp genotypes and any particular primary malaria clinical symptoms (P-value>0.05) table 8.

Table 8. The relation of the most common clinical malaria with Hp genotypes

Symptom	Hp1-1 N(%)	Hp2-1 N(%)	Hp2-2 N(%)	chix^2, p-value
Fever	8(7.2)	4(3.6)	99(89.2)	0.67, 0.716
Vomiting	5(6.3)	2(2.5)	72(91.1)	0.51, 0.773
Headache	8(7.0)	3(2.6)	104(90.4)	1.80, 0.406
Nausea	2(5.7)	0	33(94.3)	1.75, 0.416
Abdominal pain	1(4.5)	0	21(95.5)	1.13, 0.568
Diarrhea	2(10.0)	1(5.0)	17(85.0)	0.71, 0.703

P-value>0.05 not statistical significantly

The distribution Hp genotypes and hemoglobin level among children and adults

The study group showed that median of hemoglobin level is not clearly overlap with Hp genotypes among age group. That mean Hb level among age group did not differ between the Hp genotypes (P-value>0.05) figure 4.

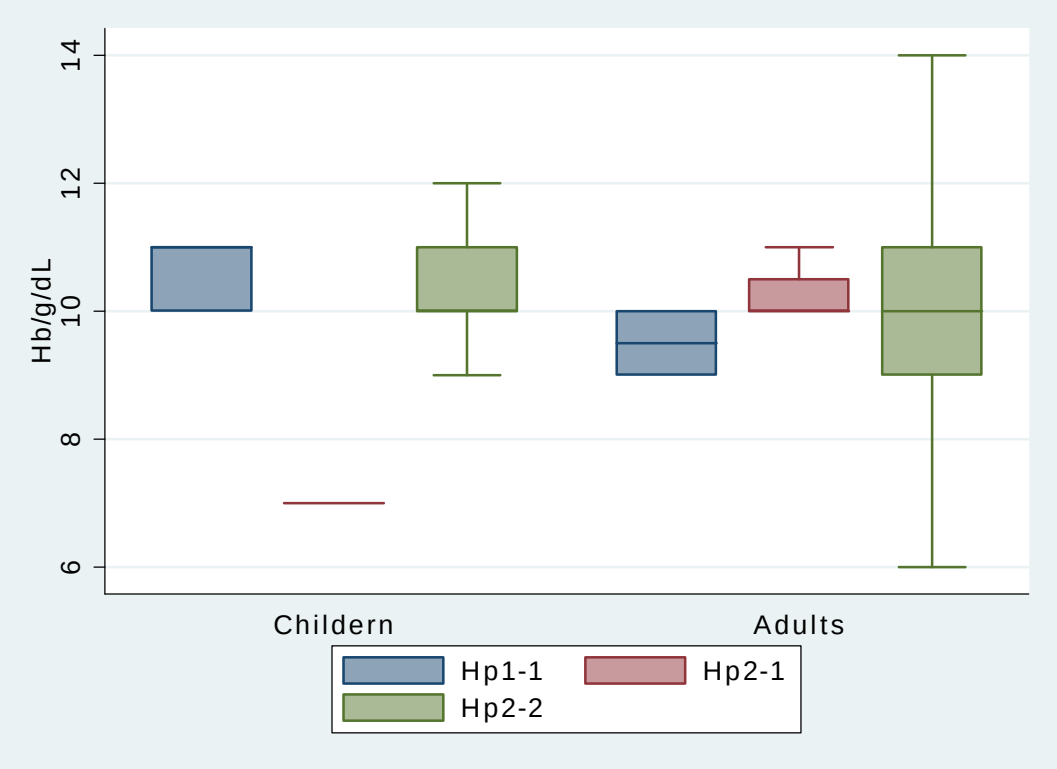

Figure 4. Box plot showing the distribution Hp genotypes and hemoglobin level among children and adults

The distribution Hp genotypes and platelet level among children and adults

In this figure, the study observed that median of platelets level is not clearly overlap with Hp genotypes among age group. That mean platelet level among age group did not differ between the Hp genotypes (P-value>0.05) figure 5.

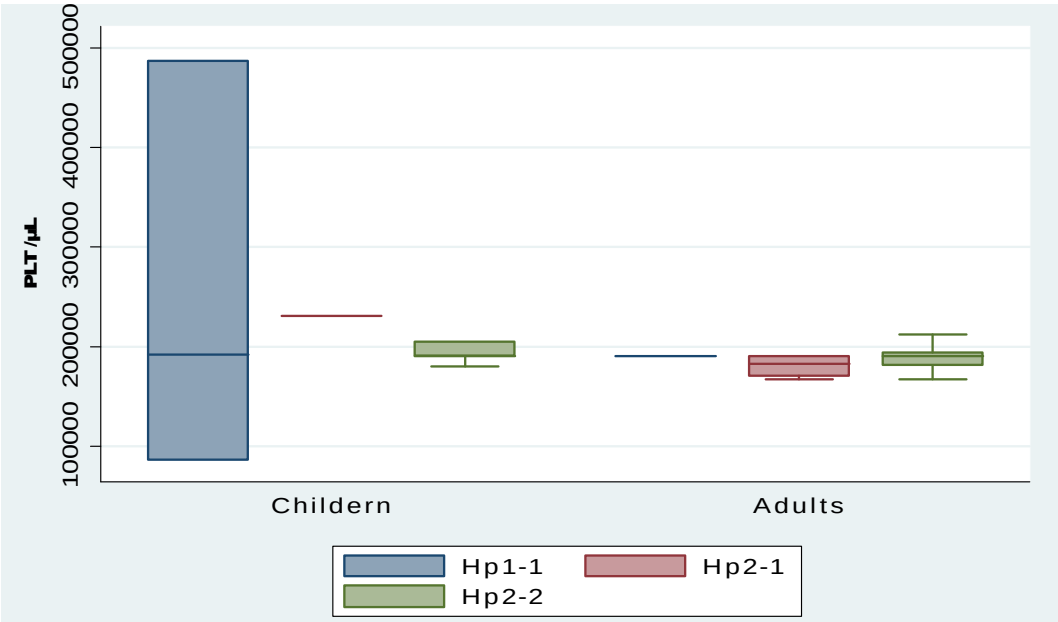

Figure 5. Box plot showing the distribution Hp genotypes and platelet level among children and adults

Discussion:

In this study it was shown that Haptoglobin genotypes prevalence, Hp1-1, Hp2-1 and Hp2-2 were found in 6.4%, 3.5% and 90.1% respectively of the patient's in Elfasher- Northern Darfur State. The overall allele frequency was 8.1% for the Hp1 allele and 91.9% for the Hp2 allele. The study observed HaptoglobinHp2-2 genotype was associated with a higher susceptibility to malaria compared to other Haptoglobin genotype.

The study done in 1998 by Elagiab in Khartoum had demonstrated the association of Hp1-1 phenotype with increased susceptibility to malaria where we found an association of Hp2-2 genotype with malaria [10]; these differences may be due to different in ethic group between the two areas and Lwanra in 2020 found No association with incidence of uncomplicated malaria with Haptoglobin genotypes in Uganda [11].

In Sudan a previous study was done by Osman et al., 2016, showed that no significant association was found between Haptoglobin phenotypes and type 2 diabetic patients [12]

Our findings on the relationship between Haptoglobin genotype Hp2-2 and frequency and susceptibility of malaria infection, Could be assuming that because the Hp2-2 hemoglobin complex is taken up more efficiently by macrophages as compared with Hp1-2 or Hp1-1, this could explain the less severe causes of malaria in this study.

In the present study the median of hemoglobin level is not clearly overlap with Hp genotypes among age group. That mean Hb level among age group did not differ between the Hp genotypes (P-value>0.05)

Interestingly, several previous studies have demonstrated that Hp2-2 genotype is associated with reduced susceptibility to malaria. In the study by Quaye et al. (2000) [13], it was found that Hp2-2 phenotype was significantly less present in malaria patients as well as in complications of malaria disease. Elagib et al. (1998) conducted a similar study in Ghana with results suggesting that the Hpl-l phenotype is associated with susceptibility to falciparum malaria and the development of severe complications. Atkinson et al. (2006) found in a study conducted in Kenya that Hp2-2 genotype was associated with reduced episodes of clinical malaria[14], . However, the study by done by Bienzle et al. (2005) [15] in northern Ghana found a limited influence of Haptoglobin genotypes to malaria susceptibility.

In view of our study and those from other studies appear that the different Haptoglobin genotypes may or may not influence reduced risk of malaria infection and development of the disease. The outcome of the relationship between Haptoglobin genotypes and disease may be influenced by disease determinants including age, ethic group and environmental factors

Conclusions

This study showed that the Hp 1–1 and Hp 2–1 genotypes each occur in nearly 4 in 10 children and the Hp 2–2 genotype occurs in 2 of every 10 children. No association with incidence of uncomplicated malaria was found. Additional studies of influence of Haptoglobin genotypes on *P. falciparum* malaria severity are needed to understand the role of these genotypes in malarial protection

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