

Original Research Article

Association of Apolipoprotein A gene polymorphism with uncomplicated Plasmodium *Falciparum* Malaria in Elfasher City, Northern Darfur.

ABSTRACT

Aims: This study aimed to investigate the lipids profile, APOA genotype with malaria infection . It was hypothesized that the malaria parasite uses cholesterol and phospholipids from its host, resulting in a decrease in serum HDL

Study design: A cross-sectional hospital -based study.

Place and Duration of Study: The study was conducted during the transmission season between July to November 2020 in different hospitals and centers in Elfasher city.

Methodology: we included (39 men and 64 female), 57.3% were adults and 42.7% were children, *plasmodium falciparum* infection, with clinical symptoms and signs of uncomplicated malaria. Parasites density, lipids profile and APOA genotyping were assayed.

Results: The mean level of CHOL and TG was 134.7 mg/dl and 73.0 mg/dl, respectively, and the average levels of LDL and HDL are 56.6 mg/dl and 56.2 mg/dl, respectively. The G/G genotypes of APOA were identified in 94.2% of the patients compared to other APOA genotypes. The overall allele frequency for the G allele was 96.0%, and the T allele was 3.9% using the Hardy-Weinberg distribution

Conclusion: In conclusions , the lipids profile and APOA genotype were not associated with uncomplicated malaria

Keywords: Malaria, Lipids, Cholesterol, Lipoproteins, High-density lipoprotein, Low-density lipoprotein, Triglycerides.

1. INTRODUCTION

“Malaria is endemic throughout most of the tropics; ongoing transmission occurs in 85 countries and territories” [1]. “The World Health Organization (WHO) reported 247 million cases and 627 thousand deaths from malaria in 2020” [2].

“The increase in cases and deaths is due to the interruption of services due to the coronavirus disease 2019 (COVID-19) pandemic, as well as a revised method of computing the malaria burden” [2]. With the revised WHO calculation, malaria accounts for 7.8 percent of the global disease burden (rather than 4.8 percent as reported previously).

“According to WHO’s latest World malaria report, Sudan carried the heaviest burden of malaria in the Eastern Mediterranean Region in 2020, accounting for more than half of all cases (56%) and deaths (61%). The Ministry of Health attributes the rise in cases to changes in rainfall patterns, frequent flooding, population movement, and the emergence of an invasive malaria vector, *Anopheles stephensi*, among other factors” [3].

“There are several regions in the country with a higher *Plasmodium falciparum* malaria burden overall, including the central region (Khartoum area), the western and south-western region (Darfour, and south Kordofan), and the eastern regions (Sennar, White Nile, and Al-Gezira). In the South Kordofan region, malaria cases in 2019 equaled 5.5% of the state’s population”. [4]. Patients with malaria often exhibit laboratory abnormalities due to an acute phase response, but little is known about serum lipid profile changes in malaria. In 1978, Lambrecht et al. [5] reported “transient lipid profile changes in high-density lipoprotein (HDL) and very low-density lipoprotein (VLDL) in human serum are related to the lipid metabolism of the parasite. It was hypothesized that the malaria parasite uses cholesterol and phospholipids from its host, resulting in a decrease in serum HDL”.

“Apolipoprotein (APO proteins) are the lipoprotein family proteins that play key roles in transporting lipoproteins all over the body. There are nearly more than twenty members reported in the APO protein family, among which the A, B, C, E, and L play major roles in contributing genetic risks to several disorders. Among these genetic risks, the single nucleotide polymorphisms (SNPs), involving the variation of single nucleotide base pairs, and their contributing polymorphisms play crucial roles in the Apolipoprotein family and its concordant disease heterogeneity that have predominantly recurred through the years” [6].

“The Lipoprotein lipase (LPL) gene, encoding the lipoprotein lipase enzyme, is approximately 30 kilobases pairs (kb) in length and is localized on chromosome 8p22. It consists of 10 exons and 9 Intron where exons 1 to 9 have an average size of 105–276bp in contrast to the length of exon 10 which is 1948bp encoding the entire 3’-untranslated region” [7].

“LPL is an extracellular enzyme on the vascular endothelial surface that degrades circulating triglycerides in the bloodstream. These triglycerides are embedded in VLDL and in chylomicrons that travel through the bloodstream. The role of lipoprotein lipase is significant in understanding the pathophysiology of type one familial dyslipidemias, or hyperchylomicronemia, and its clinical manifestations. LPL also plays a significant role in understanding the cardiac pharmacology of fibrates as a class of medications and in the management of patients with high levels of serum triglycerides. In this review, we will explore the function, pathophysiology, and clinical relevance of lipoprotein lipase” [8].

“The triglyceride lipase gene subfamily (TLGS) is comprised of three evolutionarily related lipases: lipoprotein lipase (LPL), hepatic lipase (HL), and endothelial lipase (EL), and plays a central role in plasma lipoprotein metabolism and homeostasis” [9].

2. MATERIAL AND METHODS

Study design: Cross-sectional survey was conducted during the transmission of 2020.

Study area: The study was conducted in the different hospitals and health centers in Elfasher city in, Northern Darfur State

Study population:

Individuals attend different hospitals or health centers with signs and symptoms of malaria infection during the study period.

Inclusion criteria

Patients diagnosed with *plasmodium falciparum* malaria by blood film, and patients of all ages were included in this study.

Exclusion criteria

Co-infection with other malaria species, refusal to donate blood, and history of diabetics and obesity

Sample size:

The sample size was calculated using the single population proportion formula and considering the following assumption [10]. The prevalence of malaria infection was based study carried out by which is estimated that the prevalence in Sudan was 7.6% with a 95% confidence interval and a 5% marginal error. Finally, a total of 103 patients will be included in the study from health centres and hospitals in Elfasher city with the formula $n = 3.84 p (1-p) / (\text{precision})^2$ Proportion=0.103, precision=0.05
 $n = 3.84 * 0.103(1-0.103) / (0.05)^2 = 103$

Sampling technique:

3 ml of blood in Lithium Heparin was taken from teach patients for Lipid profile, after obtaining informed consent.

Data collection:

Age, sex, occupation, residence, malaria history, clinical data, diagnostic tests, and lipids profile was obtained using laboratory test and a questionnaire

Blood film:

A thin and thick blood smear was done using 10% Giemsa stain and examined by microscopy for species identification and parasite count. [11, 12].

Total cholesterol, triglycerides, LDL, and HDL were measured using Spectrophotometer according to the manufacturing protocol of biosystem kits. And LDL cholesterol by Friedewald's equation ($F\text{-LDL-C (mmol/L)} = TC - HDL\text{-C} - TG/2.2$) [13]

DNA extraction:

DNA extraction was purified from peripheral blood dry spots paper using a DNA extraction kit (Kogene biotech, Power prep, Korea) according to the manufacturer's protocol.

DNA quantity

The quantity of DNA was determined by U.V. spectrophotometric method (Gene Quant, Amersham, UK).

DNA amplification and detection of LPL-PvuII between exons 6 and 7

The PCR was carried out on BIO-Rad thermocycler (USA) using forward primers 5'-ATGGCACCCATGTGTAAAGGTG and reverse primers 5'-GTGAACTTCTGATAACAATCT -3' [14], and the reaction mixture consisted of 4 µl Solis BIODYNE master mix, 1.0 µl from each primer, DNA 5 µl and completed to 20 µl with nuclease-free water. The following PCR conditions were used. The reaction mixture was initially denatured at 95°C for 5 min, followed by 35 cycles of 95°C for 1 min, annealing at 49.5°C for 1 min, extension at 72°C for 1 min, and final extension at 72°C for 5 mins

The PCR products were then allowed to run on 1.5% Agarose gel to check the amplification of the desired product. The ladder (100 bp) was also run along. The amplified product was 440 bp in size.

Digestion of PCR products by the PvuII

10 µl of the PCR products were added into the 2.0 µl of 10X buffer, 2.0 µl of the PvuII (Thermo scientific) enzyme, and 6.0 nuclease-free water, incubated at 37°C for 6 hours and the digested products were run on 2% agarose

Electrophoresis of PCR digestion

The digested PCR products were then electrophoresed on 1.5% Agarose gel in 1x TBE buffer and stained with 0.5 µgm Ethidium bromide for at least an hour. Products were visualized by staining with Ethidium bromide. The PvuII restriction site (Intron 6) yields 330 bp and 110 bp fragments (TT), 440, 300 and 110 bp GT genotype, and 400 bp GG genotype

3. RESULTS

Table 1: Distribution of demographic and lipid profile among patients with malaria

The 103 study subjects diagnosed with malaria, (57.3%) were adults and (42.7%) children. Their average age was 24.5 ranging from 7 days to 79 years old. The gender distribution was (62.1%) females and (37.9%) males. The clinical parameters such as CHOL and T.G were found to be in normal range, the mean was 134.7 and 73.0, respectively. The levels of LDL and HDL were observed in these patients, the average was 56.6 & 56.2, respectively. With regard to parasitemia, most of these patients (48.5%) were categorized as low. The characteristics of the study group are summarized in Table 1.

Characteristics		Patient N=103
Age group N (%)	Children	44(42.7)
	Adults	59(57.3)
Gender N (%)	Male	39(37.9)
	Female	64(62.1)
CHOL mg/dl	mean(SD)	134.7(33.3)
T.G mg/dl	mean(SD)	73.0(33.2)
LDL mg/dl	mean(SD)	56.6(29.7)
HDL mg/dl	mean(SD)	56.2(34.3)
Parasite density N (%)	Low(+)	50(48.5)
	Intermediate(++)	39(37.9)
	slightly high(+++)	13(12.6)
	High(++++)	1(1.0)

N: number of study subjects, SD: standard deviation

Table 2: APOA genotypes and allele frequency in the study group

Three main genotypes of APOA were identified in these patients. G/G genotype was the most common genotype in these patients (94.2%) compared to other APOA genotypes. The overall allele frequency for the G allele was (96.0%), while the expected frequency of T allele was only (3.9%) using the Hardy-Weinberg distribution (table 2).

APOA genotype	Patients N=103 (%)
G/G	97(94.2)
G/T	4(3.9)

T/T	2(1.9)
Allele G	198(96.0)
Allele T	8(3.9)

Table3: Characteristics of the study population, by APOA genotype

The analysis of APOA genotypes was carried out based on age group and gender. The majority of these patients (57.7%) who were adults had higher G/G genotype compared to children group (42.3%). Only one T/T genotype was found in children and the same in the adults group. The G/G genotype in gender distribution, most of these patients was females (62.9%). None of these age and gender factors revealed statistically significant association ($P= 0.05$).

Characteristics	GG n=97	GT n=4	TT n=2	chix ² , p-value
Age group N(%)				
Children	41(42.3)	2(50.0)	1(50.0)	0.138, 0.933
Adults	56(57.7)	2(50.0)	1(50.0)	
Gender N(%)				
Male	36(37.1)	2(50.0)	1(50.0)	0.399, 0.819
Female	61(62.9)	2(50.0)	1(50.0)	

$P=0.05$

Table 4: Comparison of lipid profile level with APOA genotype

The analysis of the APOA genotypes, according to lipid profile, showed higher levels of CHOL and T.G in G/T genotype (148.0& 82.3, respectively) compared to other APOA genotypes. It showed no significant difference among these genotypes (p -value > 0.05). Lower levels of LDL and HDL were found in T/T genotype (41.0& 35.0, respectively) compared with higher levels observed in G/T genotype. These levels of LDL and HDL showed no significant difference ($p= 0.05$ table 4).

Parameter	GG	GT	TT	F-test, p-
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					value
CHOL	mean(SD)	134.3(33.4)	148.0(40.7)	124.0(0.0)	0.42, 0.657
T.G	mean(SD)	73.9(32.8)	82.3(46.7)	41.0(11.3)	3.68, 0.159
LDL	mean(SD)	56.7(30.3)	61.5(16.9)	41.0(11.3)	1.59, 0.452
HDL	mean(SD)	55.9(34.6)	74.8(26.6)	35.0(25.5)	3.88, 0.144

$P=0.05$

Table 5: Comparison of parasite counts with APOA genotype

Parasitemia	GG N=97 (%)	GT N=4 (%)	TT N=2 (%)	chix ² , p-value
Low(+)	47(48.5)	2(50.0)	1(50.0)	1.12, 0.981
Intermediate(++)	36(37.1)	2(50.0)	1(50.0)	
Slightly high(+++)	13(13.4)	0	0	
High(++++)	1(1.0)	0	0	

$P=0.05$ is not statistically significantly

The study group showed that the median LDL level does not overlap with APOA genotypes among age groups. This level of LDL showed no significant difference ($p= 0.05$).

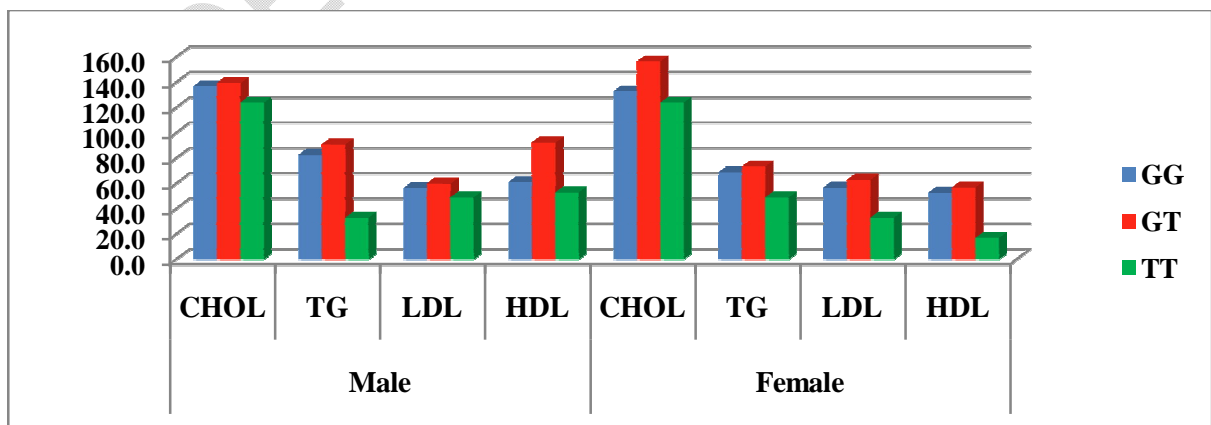


Figure 1: The frequency of lipid profile by gender among patients.

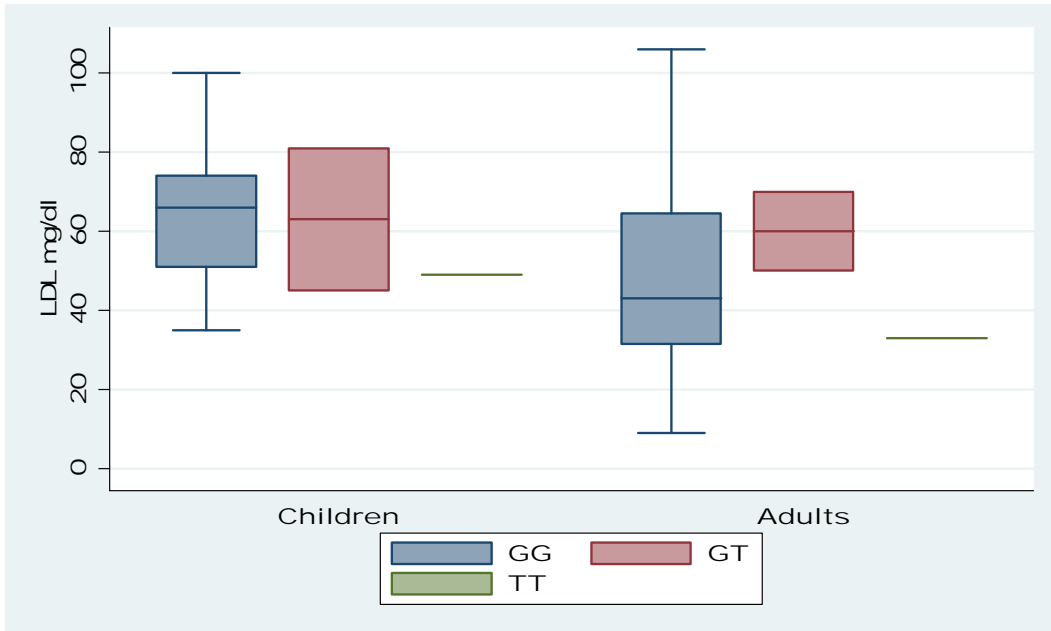


Figure 2: Box plot showing the distribution of APOA genotypes and LDL levels among children and adults

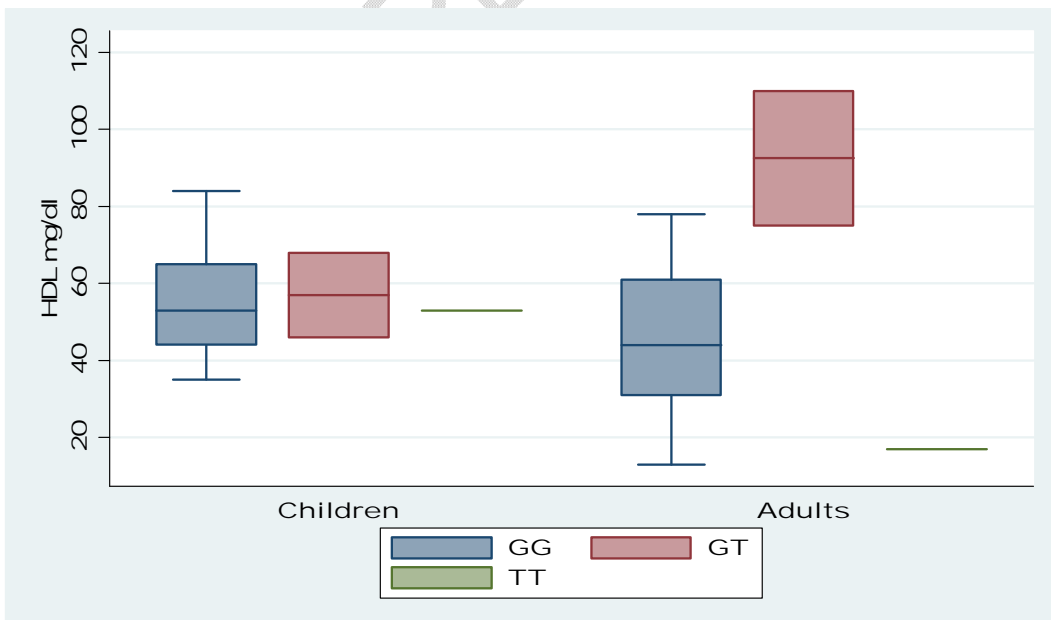


Figure 3: Box plot showing the distribution of APOA genotypes and HDL levels among children and adults

In this figure, the study observed that the median HDL level does not overlap with APOA genotypes among age groups. This level of HDL showed no significant difference ($p= 0.05$)

Statistical Analysis:

Statistical analyses were conducted using Stata version 14 (StataCorp, College Station, Texas) software. In the descriptive analysis, the visual presentation of data in tables and figures given provides demographic and clinical data in numbers, percentages, and figures as a clear indication of study population data distribution and associations. Categorical variables were reported using the chi-square test. Comparisons of continuous variables were made using the one-way analysis of variance ANOVA for parametric data and the Kruskal Wallis test for nonparametric data. The Hardy-Weinberg Equilibrium was tested for APOA genotypes and allele frequencies among patients. Then, important statistical conclusions were drawn. An alpha value of < 0.05 denoted a statistically significant difference in all statistical comparisons.

Discussion

“Changes in lipid profile are seen in many patients infected with the malaria parasite. The malaria parasite causes hepatocellular damage and disturbs lipid handling by the liver. Inside hepatocytes and erythrocytes, the parasite replicates rapidly scavenging cholesterol and lipids required for its growth and metabolism from the host. It also requires host lipids for detoxification of free heme to form the malarial pigment”. [15]

In this study, the lipids profile of patients with uncompleted plasmodium *falciparum* such as CHOL and T.G were found to be in the normal range, the mean was 134.7 mol/L and 73.0, respectively. The levels of LDL and HDL were observed in these patients, the average was 56.6 and 56.2, respectively. A study from India [16] reported triglyceride result was higher than the normal reference range in malaria, which was in disagreement with our results, while we found similar results of cholesterol and LDL levels of the study participants were within the normal reference range.

Several studies have reported a significant association between LPL gene polymorphisms and lipids profile [17]. In a study done by Megabiaw and colleagues in 2022 in Ethiopia, they found “lower HDL (87.5%) and normal LDL and TC were observed. After treatment, 100% AST, ALT, HDL, and LDL and 92% ALP, 94.3% TC, and 86.4% TG levels were in the normal range. In another study by Gurjeet Singh they found the mean level of AST and ALT increased while HDL decreased from low to higher density parasitemia”. [18]

A systematic review and meta-analysis (2013) showed that total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were lower in malaria patients compared with healthy controls, while we found HDL, TC, and LDL within normal range. This could be explained by different populations having different lipids profile [19]

The study done by Aucan in Gambia of APOE polymorphisms does not influence protection against cerebral malaria and severe malarial anemia but could play a role in protection against a particularly severe form of severe malaria. [20]

In the present study, the APOA genotype was 57.7% who were adults had the G/G genotype compared to the children group 42.3%. Only one T/T genotype was found in children and the same in the adult group. The G/G genotype was found in (62.9%) of females and 37.1 were males. None of these age and gender factors revealed a statistically significant association $P=0.05$

GG genotype of APOA lipoprotein was common in our study at 94.2%, the overall frequency for the G alleles was 96% while the T alleles were 3.9%, using the Hardy-Weinberg distribution .

The APOA genotypes, according to lipid profile, showed higher levels of CHOL and T.G in G/T genotype (148.0 and 82.3, respectively) compared to other APOA genotypes. It showed no significant difference among these genotypes (p -value > 0.05). Lower levels of LDL and HDL were found in T/T genotype (41.0& 35.0, respectively) compared with higher levels observed in G/T genotype. These levels of LDL and HDL showed no significant difference $p = 0.05$)

In Comparison of parasite counts with APOA genotype, the results of the study demonstrated the relation between APOA genotypes and parasite count. 48.5% of the G/G genotype had low parasitemia. Only one patient in this group had high parasitemia. No

association was revealed between APOA genotypes and parasite count (P-value=0.05, no statistical significance).

The average levels of CHOL and T.G among the children group were higher (156.5 & 73.5, respectively) among G/T genotype patients compared to other genotypes. Children with the T/T genotype had low LDL and HDL levels. The same can be said for the adult group. This result suggests that the APOA genotypes were not associated with clinical parameter P-value>0.05 not statistically significant.

4. CONCLUSION

Based on the findings of this investigation, it is clear that Assessment of lipid profile parameters plays an important role in effective intervention management in malaria infection.

CONSENT

All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication.

Ethical approval: Ethical approval was obtained from Ministry of health, Northern Darfur state

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ABBREVIATIONS

WHO: World Health Organization
HDL: High-density lipoprotein
VLDL: Low-density lipoprotein
APO proteins: Apolipoprotein (APO proteins)
SNPs: Single nucleotide polymorphisms
LPL: Lipoprotein lipase
DNA: Deoxyribonucleic acid
PCR: *Polymerase chain reaction*
TG: triglycerides
CHOL: *Cholesterol*
PCR: Polymerase chain reaction
RFLP: Restriction Fragments length Polymorphism