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Association between Genotype and Lipid Profile in Patients with Beta Thalassemia

Seham Fathy Azab[1], Mohamed Abdel Kader Al Malky[1], Nermin Raafat Abdel Fattah[2], Ahmed Khalil Ibrahim[1]
Pediatrics[1] and Medical Biochemistry[2] Departments, Zagazig University, Egypt
***Corresponding author:** Ahmed Khalil Ibrahim, **Email:** a.eltokhy@yahoo.com, **Mobile:** 01558226000

ABSTRACT

Background: The inherited hematological condition thalassemia is defined by a reduction in or lack of synthesis of one or more globin chains. One or more mutations in the beta-globin gene cause β thalassemia. Because of dyslipidemia, children with beta thalassemia are susceptible to early atherosclerosis. Some clinical characteristics of thalassemia, such as altered endocrine function and greater susceptibility to infections and vascular problems, may be pathologized by lipids and lipoproteins. The objective is to assess the serum lipid profiles of individuals with β thalassemia and determine any potential genetic correlations. **Subjects and methods:** The study included 73 children with β thalassemia and 73 age and sex matched children as a control group. Lipid profile was assessed in both groups. Patients genotype was analyzed by PCR. **Results:** In β thalassemia patients, total cholesterol, LDL-C, and HDL-C levels were significantly decreased while, TG concentration was significantly increased in patients compared to normal control group. 49.3% of patients had $\beta^+\beta^+$ genotype, 35.6% of them had $\beta^0\beta^0$ genotype and 15.1% had $\beta^0\beta^+$. The commonest mutation was the homozygous IVS 1–1 (19.2%) followed by homozygous IVS 1-110 (15.1%) then homozygous IVS 1-6 (11%). $\beta^0\beta^0$ genotype is correlated with more reduction in TC, and LDL-C HDL-C levels and more increase in TG level. **Conclusion:** We recommend regular monitoring of lipid profile in patients with β thalassemia, especially those with $\beta^0\beta^0$ genotype to prevent or at least enhance the early detection of cardiac disease.

Keywords: Beta thalassemia, lipid, genotype, children**.**

INTRODUCTION

Thalassemia is a hereditary hemolytic anemia caused by insufficient or nonexistent globin chain synthesis in one or more hemoglobin globulins. All thalassemia syndromes are characterized by unbalanced globin chain production, which is the result of this **(1).** With an estimated carrier incidence of 9–10%, beta thalassemia is the most prevalent chronic hemolytic anemia in Egypt **(2).** Certain clinical features of thalassemia, such as changes in endocrine function and an increased risk of infections and vascular problems, may be caused by lipids and lipoproteins **(3).**

For a considerable amount of time, it has been known that beta thalassemia is associated with abnormal lipid profiles, such as low triglycerides, low HDL-C, low LDL-C, and low total cholesterol **(3).**

Iron overload in patients with beta thalassemia results in the production of various lipid radicals and oxidized cholesteryl esters species in lipoproteins [4].

AIM OF THE WORK

Our goals are to determine the association between thalassemia patients' genotype and lipid abnormalities and to assess additional risk variables that might be involved in these lipids' modifications.

PATIENTS AND METHODS

A case control study was carried out on 73 β thalassemia children who were included during their routine follow-up appointments at Zagazig University Hospital's pediatric hematology outpatient clinic. As a control group, 73 age- and sex-matched, healthy kids were involved. A complete history, a comprehensive clinical examination, and laboratory studies were performed on all patients and controls. These investigations included: Utilization of a fully automated clinical chemistry autoanalyzer system to measure serum levels of triglycerides and total cholesterol (Konelab (201); Thermo Electron Corporation, Vantaa, Finland). **1)** Using Micro Lab 200 (Vital Scientific NV, DIERN, Netherlands), the colorimetric method was used to assess the level of high-density lipoprotein (HDL) cholesterol. Human Gesellschaft für Biochemica and Diagnostica GmbH (Wiesbaden, Germany) provided the reagents. **2)** The Friedewald equation was used to determine the LDL-cholesterol level.

3) The proteinase K procedure was used to extract the genomic DNA from the patients, and ARMS PCR and RFLP were used for analysis to determine the patients' genotype. Direct genome sequencing using an ABI 3730 DNA analyzer (Applied Biosystem Inc., Foster City, CA) was used to find unknown mutations.

RESULTS

Patients with beta thalassemia were 13.82 years old on average. 34 women and 39 men made up the group. The mean age of controls was 13.68 years, and they were 37 males and 36 females. In β thalassemia patients, In comparison to the normal control group, the patients' levels of total cholesterol, LDL-C, and HDL-C were much lower, but their TG concentration was significantly higher (Table 1). There was significant relationship between genotype (based on beta chain production) and each of total, HDL, LDL cholesterol and serum triglycerides where patients with β^0 β^0 genotype had significantly lower total cholesterol, LDL and HDL and significantly higher serum triglycerides than control group (Table 2). There was significant relationship between phenotype and each of total cholesterol, HDL, LDL cholesterol and triglycerides where total, LDL and HDL cholesterol were significantly lower and triglycerides significantly higher in patients with thalassemia major compared to those with thalassemia intermedia (Table 3). There was significant relationship between gene mutation and all of total cholesterol, HDL, LDL cholesterol and triglycerides where there was significant reduction of TC, HDL, LDL and significant increase in TG in homozygous Codon 15, followed by homozygous Codon 5, and homozygous IVS 1-1 genotypes (Table 4).

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Table 1. Comparison between the studied groups regarding lipid profile:

mg/dl	Case group (n=73)	Control group (n=73)	t	p
	Mean ± SD	Mean ± SD		
Total cholesterol	131.96 ± 23.6	168.53 ± 10.44	-12.11	**<0.001****
LDL cholesterol	72.08 ± 16.26	108.12 ± 8.66	-16.713	**<0.001****
HDL cholesterol	22.77 ± 5.02	44.08 ± 4.36	-24.505	**<0.001****
Triglycerides	152.53 ± 22.52	107.08 ± 17.65	13.574	**<0.001****

Table 2. Relationship between genotype and lipid profile among studied patients:

Genotype	Total cholesterol	LDL cholesterol	HDL cholesterol	Triglycerides
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
B^0B^0	104.69 ± 9.83	52.58 ± 5.2	15.65 ± 1.5	179.42 ± 8.71
B^0B^+	128.09 ± 6.89	70.64 ± 4.88	22 ± 1.67	155.82 ± 5.21
B^+B^+	152.83 ± 7.62	86.61 ± 4.19	28.14 ± 2.13	132.11 ± 3.42
F	250.201	400.793	338.38	460.77
p	<0.001**	<0.001**	<0.001**	<0.001**
P_1	<0.001**	<0.001**	<0.001**	<0.001**
P_2	<0.001**	<0.001**	<0.001**	<0.001**
P_3	<0.001**	<0.001**	<0.001**	<0.001**

F One way ANOVA test p1 difference between B0B0 and B0B+ p2 difference between B0B+ and B+B+ p3 difference between B0B0 and B+B+ *p<0.05 is statistically significant **p≤0.001 is statistically highly significant.

Table 3. Relationship between phenotype and lipid profile among studied patients:

Phenotype	Total cholesterol	LDL cholesterol	HDL cholesterol	Triglycerides
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
TM (n=61)	126.41 ± 21.82	68.36 ± 15.19	21.28 ± 5.43	157.13 ± 21.84
TI (n=12)	160.17 ± 3.33	91.0 ± 2.37	30.33 ± 1.23	129.17 ± 2.29
t	-11.429	-10.98	-11.604	9.734
p	<0.001**	<0.001**	<0.001**	<0.001**

t independent sample t test *p<0.05 is statistically significant **p≤0.001 is statistically highly significant

Table 4. Relationship between gene mutation and lipid profile among studied patients

Gene mutation	Total cholesterol	LDL cholesterol	HDL cholesterol	Triglycerides
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Homozygous Codon 15	99.5 ± 7.78	50.5 ± 3.54	15.5 ± 2.12	177.5 ± 10.61
Homozygous codon 37	118	64	19	178
Homozygous Codon 39	103.33 ± 11.55	52.33 ± 6.43	16.33 ± 2.08	190.67 ± 18.88
Codon 39, IVS 1-1	111.75 ± 9.61	56 ± 6.43	16.5 ± 0.58	174.25 ± 4.35
Codon 39, IVS 1-6	132.5 ± 3.54	74.0 ± 1.41	23.0 ± 1.41	147.5 ± 3.54
Codon 44, codon 27	120	60	17	170
Homozygous codon 5	100	50	15	180
Codon 5, IVS 1-110	135	73	24	157
Homozygous IVS 1-1	102.0 ± 8.85	50.79 ± 4.82	15.0 ± 1.24	179.5 ± 5.75
IVS 1-1, IVS1-1110	122.5 ± 5.0	66.25 ± 2.63	20.5 ± 0.58	159.75 ± 0.5
IVS 1-1, IVS 11-745	130.5 ± 0.71	74.0 ± 1.41	23.0 ± 0	152.5 ± 3.54
IVS 1-1, IVS 1-6	138	78	24	158
IVS 1-110, Codon 39	120	65	20	160
Homozygous IVS 1-110	144.73 ± 4.5	82.73 ± 2.45	26.27 ± 1.19	134.27 ± 2.72
IVS 1-110, IVS 1-6	153.0 ± 7.07	85.5 ± 3.54	27.5 ± 2.12	134.0 ± 4.24
IVS 1-110, IVS 11-745	153.33 ± 5.77	86.0 ± 2.65	27.67 ± 1.16	133.0 ± 1.73
IVS 1-6, IVS 1-5	160.0 ± 3.56	90.25 ± 3.86	29.75 ± 1.89	130.25 ± 4.03
Homozygous IVS 1-6	160.17 ± 2.79	90.67 ± 1.97	30.5 ± 0.84	128.67 ± 1.03
IVS 1-6, IVS 11-745	150	85	27	135
IVS 1-6, IVS 11-848	160	92	30	130
IVS 1-6, promotor 87	161.5 ± 0.71	92.0 ± 0	31.0 ± 0	128.0 ± 0
Homozygous IVS 11-745	150.5 ± 6.61	85.0 ± 3.56	27.25 ± 2.06	132.75 ± 4.27
Homozygous IVS 11-848	157	88	29	132
Homozygous promotor 87	151	85	27	135
F	36.06	65.823	62.769	48.241
p	**<0.001****	**<0.001****	**<0.001****	**<0.001****

DISCUSSION

One of the most prevalent monogenic hereditary diseases is β-thalassemia a single or more than hundreds of mutations in associated genes might be considered as the cause of this disease. as much as 200 different mutations have been recorded that affect the beta-globin gene, which are hence a cause of diverse and varying phenotypes and genotypes of the disease [5].

Lipid abnormalities have been detected in β thalassemia and in various hematological disorders. Due to dyslipidemia, children with β thalassemia are susceptible to early onset atherosclerosis [6].

In order to determine whether there are any genetic correlations, we analyzed the serum lipid profiles of individuals with β TM and β TI in this study.

The study's findings demonstrated that, in comparison to the control group, children with thalassemic disorders had considerably lower levels of total serum cholesterol, LDL, and HDL and greater levels of serum triglycerides. This is consistent with the findings published by **Hartman *et al.* [7], AI-Quobaili and Abou Asali [3], and Tselepis *et al.* [8].**

In our investigation, the Beta Thalassemic patients had low total serum cholesterol, HDLc and LDLc, along with high TGs, which was also consistent with **Ragab *et al.*[9]** findings in Egypt and Bordbar et al. in Iran [10].

Additionally, our findings concur with those of **Shalev *et al.* [11]** He stated that elevated erythropoietic activity, which raises cholesterol needs and causes iron overload-related liver damage, is one of the mechanisms behind hypocholesterolemia in thalassemia major. Additionally, the primary cause of decreased plasma cholesterol levels in patients with β thalassemia major is enhanced uptake of LDLc by reticuloendothelial system histiocytes and macrophages.

On the contrary, **Mario *et al.* [12]** found that, in comparison to controls, thalassemic individuals had greater concentrations of TG, LDL, and HDL. Their findings were explained by the possibility that the elevation in circulating TG in thalassemic individuals was caused by a decrease in extra hepatic lipolytic activity.

In contrast, there was a substantial negative association found in this study between age, HDLc, and cholesterol. A significant positive correlation was found between TG and age.

Our findings concur with those of **Flavio [13]**, who discovered a favorable relationship between age and TG levels in individuals with the predominant type of β thalassemia.

In contrast, **Kamal and Talal [14]** revealed that aging had no effect on the lipid profile in individuals with thalassemia major.

Our research revealed a substantial positive link between blood ferritin and triglycerides and a significant negative correlation between serum ferritin and total cholesterol, LDLc, and HDLc .

Serum ferritin and TG levels showed a significant positive link, while serum ferritin and total and LDLc levels showed a negative correlation that was not statistically significant, according [15].

Kadhim *et al.* [16] discovered that in children with β TM, TG has a positive connection with serum ferritin, which is consistent with the findings published by **Sherief *et al.* [17].**

Ragab *et al.* [9] discovered that there was no meaningful relationship between serum ferritin and lipid profiles.

This study found a substantial correlation between each of total cholesterol, HDLc, LDLc, and triglycerides and phenotype (almost 84% of patients had phenotypic TM; 61 TM and 12 TI).

Patients with TI have significantly higher total cholesterol, LDL and HDL cholesterol, but significantly lower triglycerides than TM .

This finding is consistent with **Bordbar *et al.*** [10], who discovered that TM patients had higher triglyceride concentrations than TI patients.

This result is in contrast with that of **Haghpanah *et al.* [18]** who found no significant difference between TM and TI in lipid profiles.

Of the patients in our study, 35.6% had the β0β0 genotype and 49.3% had the β+β+ genotype. Homozygous IVS 1-110 IVS 1-110 (15.1%), homozygous IVS 1-6 IVS 1-6 (11%), and homozygous IVS 1-1 IVS 1-1 (19.2%) were the most prevalent mutations.

These results agree with the study of **Hassan *et al.* [19]** and concur with other earlier Egyptian research. Although the most common mutations in the majority of Egyptian research were IVS 1–1(G>A), IVS 1–110(G>A), and IVS 1–6(T>C), the order of frequency of these mutations varies in various investigations [20-22].

We noticed significant reduction in total cholesterol, HDLc, LDLc and significant increase in TG in $\beta^0\beta^0$ compared to $\beta^0\beta^+$ genotype.

This result agrees with **Madani *et al.* [23]** they found that, when compared to homozygous β+ type mutations, severe β0 type mutations were significantly associated with reduced LDLc levels, significantly lower levels of total cholesterol, and non-significantly lower concentrations of TG.

This study showed significant relation between gene mutation and all of TC, HDLc, LDLc and triglycerides., there is significant reduction of TC, HDLc, LDLc and significant increase in TG in homozygous mutation Codon 15, followed by homozygous Codon 5, and homozygous IVS 1-1 mutations

In the study of **Bordbar *et al.* [10]** demonstrated that homozygous IVS II-1, the most severe genotype, linked with a greater decrease in TC and LDLc concentrations than did the IVS II-1/IVS I-110 and homozygous IVS I-5 groupings.

CONCLUSION

We concluded that β thalassemia patients had a disturbed lipid profile in the form of lower total cholesterol, LDL-C, and HDL-C levels and higher Triglycerides concentration. Additionally, there was a significant relationship between the inherited genotype and lipid profile in these patients where $\beta^0\beta^0$ genotype was associated with more reduction in TC, HDL-C and LDL-C levels and more increase in TG, which may contribute to the development of atherosclerosis and cardiac diseases in patients with β thalassemia.

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