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Predicting Factors of Atherosclerosis in Children with Beta-Thalassemia Major

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Abstract

Background: Atherosclerosis is an important cardiovascular disorder in beta-thalassemia major patients. The present study aimed to predict factors of atherosclerosis in children with beta-thalassemia major.

Materials and Methods: This cross-sectional study was conducted on 36 patients with beta-thalassemia major and 36 healthy children as the control group. The carotid intima-media thickness (CAIMT) and osteoprotegerin (OPG) were compared between groups.

Results: The mean age of the patients in the case and control groups were 13.5 ± 3.7 and 13 ± 3.1 years old, respectively. Significant differences in CAIMT in the right (P = 0.001) and left (P = 0.001) arteries were recorded between the two groups. The mean serum OPG in the beta-thalassemia group was significantly higher than the control group (3.9 ng/ml and 1.4 ng/ml, respectively, P=0.001).

Conclusion: CAIMT is a non-invasive method for diagnosing atherosclerosis. According to the significant difference between groups, serum OPG could be helpful in the diagnosis of early atherosclerosis in beta-thalassemia major.

Key words: Atherosclerosis, Beta-Thalassemia, Osteoprotegerin

Introduction

Thalassemia is a heterogeneous group of diseases caused by the absence or decrease in one or more chains in the globin subunit of the adult hemoglobin structure (1). Beta-thalassemia major as the result of nearly complete loss of beta-globin chain production causes severe anemia that requires regular blood transfusions throughout life (2). Besides anemia, patients with beta-thalassemia major are confronted with problems due to the disease itself and its complications. Atherosclerosis is a critical cardiovascular complication in beta-thalassemia major patients (3). Different factors are implicated in its pathogenesis such as iron overload, pro-inflammatory environment, and endothelial dysfunction. Because of the advancements in the blood transfusion protocols, better management of complications, the life expectancy of beta-thalassemia major patients has increased, and most patients enter adulthood. Iron overload is an unfavorable consequence of transfusion therapy, and complications mainly occur due to chronic iron accumulation and involve the heart, liver, and other organs. Iron overload causes early and accelerated atherosclerosis due to endothelial dysfunction through oxidative damage (4-7). Studies showed that the measurement of carotid arterial intima-media thickness (CAIMT) is a suitable non-invasive diagnostic procedure for detecting subclinical atherosclerosis. Other studies have shown that CAIMT predicted many atherosclerosis-related events, including stroke and myocardial infarction (8-9). Serum osteoprotegerin

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(OPG) is among the new circulating biomarkers of atherosclerosis. OPG is a glycoprotein consisting of 401 amino acids and is an inhibiting factor of osteoclastogenesis (10). OPG is related to Von Willebrand factor and Weibel-Palade bodies in the endothelial cells. In vitro, tumor necrosis factor (TNF) or interleukin-1 stimulates the secretion of the OPG-Von-Willebrand factor complex. Pro-inflammatory cytokines activate endothelial cells and are responsible for the circulating OPG in subjects with atherosclerosis (11). Moreover, vascular smooth muscles are the primary origin of increased serum OPG in patients with cardiovascular disease (12). The previous study suggested that OPG was induced by atherosclerosis and limited vascular calcification (13). Although OPG can inhibit arterial calcification, it cannot reverse it (14); therefore, the current study aimed to assess the serum OPG as an early biomarker for atherosclerosis in patients with beta-thalassemia major.

Materials and Methods

Design and settings

This cross-sectional study with a control group was conducted in 17 Shahrivar Children Hospital, Iran, from March to February 2018. Thirty–six patients with beta-thalassemia major (5–19 years old) and thirty–six healthy children (control group) were enrolled in this study based on their clinical history and laboratory examinations. Patients with beta-thalassemia referred to the clinic of 17 Shahrivar hospital were assessed. Controls were children referred to the clinic for routine visits. Groups were matched by age and sex.

Inclusion and exclusion criteria

This study included 5-19-year-old children of both sexes. The case group included children diagnosed as beta-thalassemia major based on the following criteria: hemoglobin (Hb) < 7 gr/dl, High HbF, absent or very low HbA, and more than eight transfusions per year. Patients with risk factors for atherosclerosis, history of hepatic disease, renal failure, cardiac diseases, diabetes mellitus, other endocrinopathies, thyroid dysfunction, smoking, OPG**,** hemoglobinopathies, hereditary hypercholesterolemia, or positive family history of premature atherosclerosis, as well as patients with human immunodeficiency virus infections, and other autoimmune or systemic disease were excluded. Controls were children referred to the clinic for routine visits.

Paraclinical assessments

The enzymatic method determined the serum lipid profile (Hitachi, Japan). Laboratory tests for detecting lipid profile and OPG required 12 hours fasting. Ferritin was assessed by *the chemiluminescence (CL) method (Abbot, USA).* The used kits were from the Pishgaman company. All blood samples were collected the day before the red blood cell (RBC) transfusion. Lipid profile assessment included; total serum cholesterol (TC), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein–cholesterol (LDL-C), and serum triglyceride (TG). Very low-density lipoprotein–cholesterol (VLDL-C) was calculated by dividing TG by 5. In the OPG assay, plasma was separated and stored at −20 °C. The OPG evaluation was performed using Human OPG ELISA kit (Hangzhou East Biopharm Co. Ltd, China). Determination of CAIMT was done by color Doppler ultrasonography. An experienced radiologist performed CAIMT measurements for all subjects. He was unaware of the clinical and laboratory results.

Statistical analysis

All data were analyzed using the IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp. Variables were mentioned as mean ± SD or number and percent. The T, Mann–Whitney U, and Chi-square tests were used to compare the results. Pearson and Spearman correlation coefficients were used. $P < 0.05$ was noted as the significance level.

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Ethical Considerations:
This study was approved by the Ethics Committee of the Vice-Chancellor of Research at Guilan University of Medical Sciences (Num: IR.GUMS.REC.1397.417, Date: 2019-01-19). Parents were informed about the study procedure and written informed consent was obtained from the parents of all participants.

Results

In this cross-sectional study with a control group, 36 patients with beta-thalassemia major and 36 healthy children in the control group were compared. The mean age in the patients with beta-thalassemia major and control groups was 13.5 ±3.7 and 13.0 ±3.1 years old, respectively (ranging between 5 and 19 years), and 52.8% of subjects were male, and 47.2% were female. The body mass index (BMI) differed significantly between groups (Table I). TC (100 ± 36 vs. 130 ± 33, P = 0.001), LDL-C (53 ± 22 vs. 75 ± 18, P =0.001), and HDL-C (32 ± 11vs. 41 ± 6, P =0.001) were significantly lower in beta-thalassemia major patients. However, TG (113 ± 42 vs. 116 ± 45, P = 0.757) and VLDL-C (22 ± 8 vs. 23 ± 9, P = 0.685) were not significantly different between groups (Table II and Fig 1). Results revealed significantly higher OPG levels in beta-thalassemia major patients compared to the control group (3.9 ± 3.1 vs. 1.4 ± 0.5ng/ml, p = 0.001) (Fig. 2). There was no significant correlation between serum OPG and other variables such as age, sex, BMI, TG, TC, LDL-C, VLDL-C, HDL-C, and CAIMT in the beta-thalassemia major patients (Table IV). However, there was a significant correlation between serum OPG and age (P = 0.015), TG (P = 0.005), and TC (P = 0.004) in the control group. Still, there was no significant correlation between serum OPG and sex, BMI, LDL-C, VLDL-C, HDL-C, and IMT at the right and left carotid arteries. No significant correlation was observed between serum OPG levels and CAIMT of both carotid arteries (Right carotid: r = 0.093, p = 0.591 and Left side: r = 0.021, p = 0.901). The CAIMTs for both sides were significantly different between groups (Right carotid: 0.53 ± 0.04 and 0.34 ± 0.06 mm, respectively p=0.001 and left carotid: 0.53 ± 0.05 and 0.35 ± 0.05 mm respectively, p=0.001) (Table III). Furthermore, there was no significant correlation between the IMT of the right carotid artery (CAIMTR) and the IMT of the left carotid artery (CAIMTL) and other variables in the beta-thalassemia major patients (Table V). On the other hand, in the control group, there was a significant correlation between CAIMTR with age (P = 0.004), LDL-C (P = 0.002), and TG (P = 0.01), but not with other variables. Also, there was a significant correlation between CAIMTL and age (P = 0.001), LDL-C (P = 0.02), and TC (P = 0.01) in the control group but not with other variables.

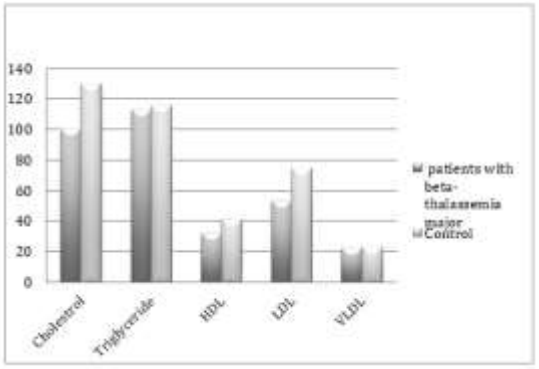

Figure 1. Lipid profile distribution of the studied groups

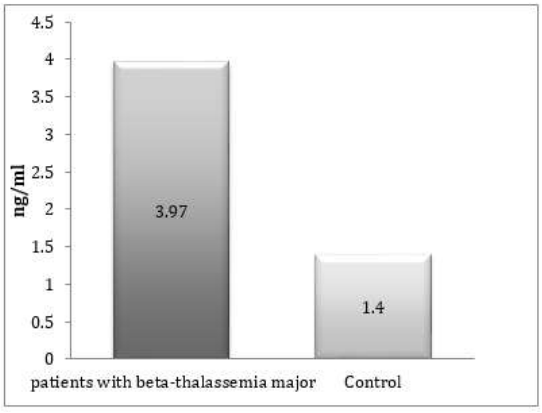

Figure 2. Serum Osteoprotegerin (ng/ml) distribution of the studied groups

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Table I: Demographic data of the studied groups

Variables	patients with beta-thalassemia major N=36	control N=36	Test	P-value
age Mean±SD Range	13.5±3.7 (5-19)	13±3.1 (6-18)	Independent T-test	0.566
sex Male Female	17(47%) 17(47%)	17(47%) 17(47%)	Independent T-test	0.005
BMI	18±0.7)16-21(	18±0.7 (16-33)	Independent T-test	0.001

Body Mass Index (BMI)

Table II: Lipid profile in the patients with beta-thalassemia major and control groups

Lipid profile	Mean±SD		P value
	Patients with beta-thalassemia major	control	
TG mg/dl	113±42	116±45	0.757
TC	100±36	130±33	0.001
LDL	53±22	75±18	0.0001
VLDL	22±8	23±9	0.658
HDL	32±11	41±6	0.0001

Triglyceride (TG), Total cholesterol (TC), Low-density lipoprotein (LDL), Very Low density lipoprotein (VLDL), High-density lipoprotein (HDL)

Table III: Radiological indices of the groups

CAIMT	patients with beta-thalassemia major N=36	Control N=36	Test	P-value
Right Mean±SD	0.53±0.04	0.34±0.06	Mann-Whitney	0.001
Left Mean±SD	0.53±0.05	0.35±0.05	Mann-Whithney	0.001

*CAIMT: Carotid artery intima media thickness

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Table IV: Correlations between OPG and different indices in the patients with beta-thalassemia major

Variables	r (correlation coefficient)	OPG P-value
age	0.010	0.955
sex	-0.087	0.613
BMI	0.074	0.668
TG	-0.007	0.969
TC	0.032	0.854
LDL	0.026	0.879
VLDL	0.009	0.957
HDL	0.103	0.550
CAIMTR	0.093	0.591
CAIMTL	0.021	0.901
Ferritin	-0.294	0.081

Body mass index (BMI), Triglyceride (TG), Total cholesterol (TC), Low-density lipoprotein (LDL), Very Low density lipoprotein (VLDL), High-density lipoprotein (HDL), the IMT of the right carotid artery (CAIMTR), the IMT of the left carotid artery (CAIMTL), Osteoprotegerin (OPG)

Table V: Correlations between carotid artery intima-media thickness and different indices in the patients with beta-thalassemia major

Variables	Right CAIMT		Left CAIMT	
	r	P-value	r	P-value
age	-0.095	0.580	-0.135	0.432
sex	0.008	0.962	-0.127	0.461
BMI	0.015	0.931	-0.065	0.707
TG	-0.149	0.387	-0.018	0.917
TC	-0.058	0.739	0.164	0.339
LDL	0.115	0.504	0.308	0.068
VLDL	-0.149	0.386	-0.013	0.940
HDL	0.027	0.878	0.281	0.097
OPG	0.093	0.591	0.021	0.901
Ferritin	0.089	0.607	0.045	0.795

Body mass index (BMI), Triglyceride (TG), Total cholesterol (TC), Low-density lipoprotein (LDL), Very Low density lipoprotein (VLDL), High-density lipoprotein (HDL), Osteoprotegerin (OPG)

Discussion

Our study showed that serum TC, LDL, and HDL were lower in the beta-thalassemia major patients and the mean serum OPG was higher in the beta-thalassemia major patients. Significant differences in IMT were recorded between the two groups. Beta-thalassemia major patients are at risk for vascular complications due to iron overload and a pro-inflammatory environment (15). Endothelial dysfunction increases arterial thickness and is noted as a significant risk factor for atherosclerosis (16). High-resolution ultrasound techniques are non-invasive assessment tools for vascular dysfunction especially in pediatric patients. Several studies have reported that CAIMT is a good prognostic factor of subclinical atherosclerosis (17). Other studies have shown that CAIMT predicts many atherosclerosis-related events,

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including stroke and myocardial infarction (8-9). Therefore, we used this technique for the early detection of atherosclerosis due to its quantitative nature and validated prediction. In line with our study, Adly et al. evaluated vascular dysfunction in young beta-thalassemia major patients and its relation to cardiovascular complications. They observed that CAIMT was significantly higher in the patients than control group (16). Furthermore, assessment of CAIMT in children with beta-thalassemia major revealed consistent results (18). Our findings suggested the risk of premature atherosclerosis in these patients based on CAIMT measurements, which was similar to other studies (19-20).

There is limited evidence about the risk of atherosclerosis in patients with beta-thalassemia major. Therefore, we assessed clinical and laboratory parameters that may be related to vascular damage and atherosclerosis. Beta-thalassemia major patients are at an increased risk for premature atherosclerosis due to dyslipidemia. The pathophysiology of lipid abnormality is not fully elucidated. Several studies assessed the lipid profile, but the results were inconsistent (21-22). The present study showed lower TC, LDL-C, and HDL-C levels in the patients with thalassemia major compared to the control group. In a consistent study by Ashar et al. findings revealed that beta-thalassemia major patients had lower serum TC, LDL-C, and HDL–C levels and raised serum TG levels compared to controls (23). Similar to our study, most previous studies reported lower TC, LDL-C, and HDL-C levels in beta-thalassemia major patients (22, 25). According to earlier reports, the circulating LDL-C in beta-thalassemia major patients is susceptible to oxidation due to increased iron levels. Macrophages and histiocytes of the reticuloendothelial system uptake oxidized LDL to form foam cells. Foam cell formation is the initial step in developing atherosclerotic plaque (24). Increased serum iron could lead to free-radical reaction and LDL oxidation. However, this process requires the depletion of antioxidant defense and a decrease in HDL-C. Our study showed no significant difference regarding TG in groups. Some studies showed elevated TG, while other studies' findings (23-24) were similar to ours. Amendola et al. assessed the lipid profile in beta-thalassemia intermedia patients and found that serum TG level was not significantly different between patients and control groups (24). Furthermore, Haghpanah et al. found no significant differences in TG levels between patients and healthy controls (23).

Different mechanisms are involved in the pathogenesis of lipid profile abnormalities in beta-thalassemia major patients, including accelerated erythropoiesis, increased TC uptake by the reticuloendothelial system, iron overload, and impaired liver function (25). In this study, we found that OPG was higher in patients with beta- thalassemia compared to the control group. Consistent with our results, Sherief et al. showed that serum OPG increased significantly in thalassemia patients compared to healthy subjects (26). Higher serum OPG was proposed as a marker for arterial injury and a predictor for coronary artery diseases and cardiovascular risk (27). Extensive cohort studies have validated the OPG assay in predicting atherosclerosis and coronary artery diseases (28-29) and supported its clinical application for predicting atherosclerosis in asymptomatic high-risk patients (30). Higher levels of serum OPG in beta-thalassemia major patients compared to controls highlighted its essential role as a predictive biomarker for subclinical atherosclerosis in this high-risk group. Furthermore, no significant correlation was found between serum OPG and CAIMT. This finding could be due to the limitations of the current study, including a smaller sample size compared to similar studies, scarcity of similar and comparable studies, and presence of confounding factors, including non-prospective study design, age diversity in

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both groups, failure to measure multiple and sequential OPG level, lack of specific relationship between OPG level and premature atherosclerosis, and lack of simultaneous measurement of other influential factors like RANKL, as well as failure to assess the osteoporosis status of the patients and finally the effect of hormonal effects on OPG metabolism in interpreting this relationship. The strength of this study was assessing a novel, simple, and non-invasive method entitled OPG as a possible biomarker for diagnosing early atherosclerosis in patients with beta-thalassemia major. Our limitation can be the few samples assessed in this study regarding the high inflation. Therefore, further multi-center studies with a larger sample size can be recommended.

Conclusion

Our study findings revealed that an impaired lipid profile combined with higher CAIMT and OPG in these patients, could be a predictor of early atherosclerosis in patients with the beta-thalassemia major. Therefore, further studies on these factors can help us better understand this relationship.

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Conflict of interest

The authors declare no conflict of interest.

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