# 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  $13.5 \pm 3.7$  and  $13 \pm 3.1$  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 regular blood transfusions requires 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 Different patients (3). 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 betathalassemia 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 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

(OPG) is among the new circulating biomarkers of atherosclerosis. OPG is a glycoprotein consisting of 401 amino acids inhibiting factor and is an 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. Proinflammatory 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 limited and 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 betathalassemia 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, hemoglobinopathies, smoking, OPG, hypercholesterolemia, hereditary 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 assessed was 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 included; total assessment serum cholesterol (TC), high-density lipoproteincholesterol (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  $\pm$  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.

#### **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  $13.0 \pm 3.1$  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  $\pm$  36 vs. 130  $\pm$  33, P = 0.001), LDL-C (53  $\pm$  22 vs. 75  $\pm$  18, P =0.001), and HDL-C ( $32 \pm 11$ vs.  $41 \pm 6$ , P =0.001) were significantly lower in betathalassemia major patients. However, TG  $(113 \pm 42 \text{ vs.} 116 \pm 45, P = 0.757)$  and VLDL-C  $(22 \pm 8 \text{ vs. } 23 \pm 9, P = 0.685)$ 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  $\pm$  3.1 vs. 1.4  $\pm$ 0.5 ng/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, significant correlation there was no 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 = 0.591and Left side: r = 0.021, p = 0.901). The CAIMTs for both sides were significantly different between groups (Right carotid:  $0.53 \pm 0.04$  and  $0.34 \pm 0.06$  mm, respectively p=0.001 and left carotid: 0.53  $\pm$  0.05 and 0.35  $\pm$  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 betathalassemia 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 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

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

#### Table I: Demographic data of the studied groups

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	control		
	beta-thalassemia			
	major			
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), Highdensity 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

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

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

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

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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 betathalassemia major patients and the mean serum OPG was higher in the betathalassemia 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). Highresolution ultrasound techniques are noninvasive assessment tools for vascular especially pediatric dysfunction in patients. Several studies have reported that CAIMT is a good prognostic factor of subclinical atherosclerosis (17). Other studies have shown that CAIMT predicts atherosclerosis-related many 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 relation cardiovascular its to 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 betathalassemia 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 lipid profile in beta-thalassemia the 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 beta-thalassemia in major patients, accelerated erythropoiesis, including increased TC by uptake 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 coronary artery diseases for and cardiovascular risk (27). Extensive cohort studies have validated the OPG assay in predicting atherosclerosis and coronary artery diseases (28-29) and supported its application clinical for predicting atherosclerosis in asymptomatic high-risk patients (30). Higher levels of serum OPG beta-thalassemia major patients in 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 nonprospective study design, age diversity in

both groups, failure to measure multiple and sequential OPG level, lack of specific relationship between OPG level and premature atherosclerosis, and lack of measurement simultaneous 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 maior. 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 betathalassemia 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.

# References

1.Farashi S, Harteveld CL. Molecular basis of  $\alpha$ -thalassemia. Blood Cells Mol Dis 2018; 70: 43-53.

2.Muncie H, Campbell J. Alpha and beta thalassemia. Am Fam Physician 2009; 80(4): 339-344.

3.Jindal G, Chavan P, Kaur R, Jaswal S, Singhal KK, Palta A, et al. Carotid intimamedia thickness and oxidative stress markers for assessment of atherosclerosis in children with  $\beta$  thalassemia major. Thalassemia Reports 2016 ; 6(1):4939-4942.

4.Qayyum R, Schulman P. Iron and atherosclerosis. Clin cardiol 2005; 28(3): 119-122.

5.Cheung Y, Ha S, Chan G. Ventriculovascular interactions in patients with beta thalassaemia major. Heart (British Cardiac Society) 2005; 91(6): 769-773.

6.Cheung Y, Chow P, Chan G, Ha S. Carotid intima-media thickness is increased and related to arterial stiffening in patients with beta-thalassaemia major. Br J Haematol 2006; 135(5): 732-734.

7.Hahalis G, Kremastinos D, Terzis G, Kalogeropoulos A, Chrysanthopoulou A, Karakantza M, et al. Global vasomotor dysfunction and accelerated vascular aging in beta-thalassemia major. Atherosclerosis 2008; 198(2): 448-457.

8.Qu B, Qu T. Causes of changes in carotid intima-media thickness: a literature review. Cardiovascular ultrasound 2015; 13(1):1-10.

9.Finn A, Kolodgie F, Virmani R. Correlation between carotid intimal/medial thickness and atherosclerosis: a point of view from pathology. Arterioscler Thromb Vasc 2010; 30(2):177-181.

10. Konstantino Y, Wolk R, Terra S, Nguyen T, Fryburg D. Non-traditional biomarkers of atherosclerosis in stable and unstable coronary artery disease, do they differ? Acute cardiac care 2007; 9(4): 197-206.

11.Aoki A, Murata M, Asano T, Ikoma A, Sasaki M, Saito T, et al. Association of serum osteoprotegerin with vascular calcification in patients with type 2 diabetes. Cardiovasc Diabetol. 2013;12:11-15.

12.Dekker M, Waissi F, Silvis M, Bennekom J, Schoneveld A, de Winter R, et al. High levels of osteoprotegerin are associated with coronary artery calcification in patients suspected of a chronic coronary syndrome. Scientific reports 2021;11(1):1-10.

13.Kaden J, Bickelhaupt S, Grobholz R, Haase K, Sarikoc A, Kilic R, et al. Receptor activator of nuclear factor kappaB ligand and osteoprotegerin regulate aortic valve calcification. J Mol Cell Cardiol 2004; 36(1):57-66.

14.Morony S, Tintut Y, Zhang Z, Cattley D. RC. Dwyer Van G, et al. osteoprotegerin inhibits vascular calcification without affecting atherosclerosis in ldlr(-/-)mice. Circulation 2008:117(3):411-420.

15.Paul A, Thomson VS, Refaat M, Al-Rawahi B, Taher A, Nadar SK. Cardiac involvement in beta-thalassaemia: current treatment strategies. Postgraduate Medicine 2019;131(4):261-7.

16.Adly AA, El-Sherif NH, Ismail EA, El-Zaher YA, Farouk A, El-Refaey AM, et al. Vascular dysfunction in patients with young beta-thalassemia: relation to cardiovascular complications and subclinical atherosclerosis. Clin Appl Thromb Hemost 2015; 21(8):733-44.

17.Bertoluci M, Cé G, da Silva A, Wainstein M, Boff W, Puñales M. Endothelial dysfunction as a predictor of cardiovascular disease in type 1 diabetes. World J Diabetes 2015; 6(5): 679-692.

18.Tantawy A, Adly A, El Maaty M, Amin S. Subclinical atherosclerosis in young beta-thalassemia major patients. Hemoglobin 2009; 33(6):463-474.

19.Dogan M, Citak E. The evaluation of carotid intima-media thickness in children with beta-thalassaemia major. Cardiol Young 2012; 22(1):79-83.

20.Merchant R, Chate S, Ahmed J, Ahmad N, Karnik A, Jankaria B. Evaluation of carotid artery dynamics & correlation with cardiac & hepatic iron in  $\beta$ -thalassaemia patients. Indian J Med Res 2016; 143(4):443-448.

21.Hassanin A, Gindi H, Wakeel M, Kassas G, Amer A. Disturbances of lipid profile and serum ferritin levels in thalassemic children. Curr Sci Int 2015; 4(2):1781-1783.

22.Morris C, Kim H, Klings E, Wood J, Porter J, Trachtenberg F, et al. Dysregulated arginine metabolism and cardiopulmonary dysfunction in patients with thalassaemia. Br. J. Haematol 2015; 169(6):887-898.

23.Haghpanah S, Davani M, Samadi B, Ashrafi A, Karimi M. Serum lipid profiles in patients with beta-thalassemia major and intermedia in southern Iran. J Res Med Sci 2010; 15(3):150-154.

24.Amendola G, Danise P, Todisco N, D'Urzo G, Di Palma A, Di Concilio R. Lipid profile in beta-thalassemia intermedia patients: correlation with erythroid bone marrow activity. Int. J. Lab. Hematol 2007; 29(3):172-176.

25.Shalev H, Kapelushnik J, Moser A, Knobler H, Tamary H. Hypocholesterolemia in chronic anemias with increased erythropoietic activity. Am. J. Hematol 2007; 82(3):199-202.

26.Sherief L, Dawood O, Ali A, Sherbiny HS, Kamal NM, Elshanshory M, et al. Premature atherosclerosis in children with beta-thalassemia major: New diagnostic marker. BMC Pediatr 2017; 17(1):69-72.

27.Nybo M, Rasmussen LM. The capability of plasma osteoprotegerin as a predictor of cardiovascular disease: a systematic literature review. Eur. J. Endocrinol 2008; 159 (5):603-688.

28.Semb AG, Ueland T, Aukrust P, Wareham NJ, Luben R, Gullestad L, et al. Osteoprotegerin and soluble receptor activator of nuclear factor-kappaB ligand and risk for coronary events: a nested casecontrol approach in the prospective EPIC-Norfolk population study 1993-2003. Arterioscler. Thromb Vasc 2009; 29(6):975-980.

29.Mogelvang R, Pedersen SH, Flyvbjerg A, Bjerre M, Iversen AZ, Galatius S, et al. Comparison of osteoprotegerin to traditional atherosclerotic risk factors and high-sensitivity C-reactive protein for diagnosis of atherosclerosis. Am. J. Card 2012; 109(4):515-520.

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30.Abdelsamei HA, El-Sherif AM, Ismail AM, Abdel Hakeem GL. The Role of the Carotid Doppler Examination in the Evaluation of Atherosclerotic Changes in beta-Thalassemia Patients. Mediterr. J Hematol Infect Dis 2015; 7(1):e2015023e20115027.