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ORIGINAL STUDY

Assessment of Serum Asymmetric Dimethylarginine Level in Children with Beta-thalassemia

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Abstract

Objective: **The aim was to evaluate serum asymmetric dimethylarginine (ADMA) level in pediatric patients who received a diagnosis of beta-thalassemia as a predictor of endothelial dysfunction.**

Background: **Beta-thalassemia is one of most common global autosomal recessive disorders. It causes poor growth and skeletal abnormalities along with hemolytic anemia that regularly involves long-term blood transfusions.**

Methods: **This cross-sectional study included 60 children divided equally in two groups. The patient group was previously diagnosed with beta-thalassemia major (β-TM), and the control group was healthy subjects who were age, sex, and socioeconomically matched with the patient group. Both groups were evaluated regarding complete blood count, liver and kidney function tests, ADMA, and carotid intima−media thickness.**

Results: **The most prevalent clinical presentation of β-TM was pallor and jaundice. The authors reported a statistically significant difference regarding hemoglobin level, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution width, platelet, white blood cells, serum ferritin, alanine transferase, and aspartate transferase between cases and controls. In addition, we observed a statistically significantly higher mean carotid intima−media thickness among the patient group for right and left sides ($P = 0.012$). Serum ADMA also showed a statistically significantly increased levels for the patient group ($P = 0.001$), and the best-detected cutoff point was 10 458.5 ng/l with a sensitivity of 96.7% and a specificity of 76.7%.**

Conclusion: **Major endothelial dysfunction is responsible for β-TM cardiovascular complications, and serum level of ADMA may be used as an early marker for endothelial dysfunction.**

Keywords: Asymmetric dimethylarginine, Beta-thalassemia, Endothelial function

1. Introduction

Beta-thalassemia is a genetic blood disorder caused by a mutation in *beta globin* gene resulting in excess free alpha globin chains, leading to destruction of red blood cells and subsequent anemia [1].

Beta-thalassemia is a highly heterogeneous group of genetic defects leading to decreased or absent β-globin production [2]. Insufficient β-globin production results in the accumulation and precipitation of unpaired α-globin chains, leading to hemolysis and ineffective erythropoiesis, resulting in severe anemia and a series of secondary complications, such as skeletal abnormalities, splenomegaly, and growth defects [3].

Iron overload attributed to recurrent blood transfusion is the primary reason for long-term complications that include damage of parenchymal organs and cardiovascular system [4]. Among these cardiovascular complications, heart failure still represents the leading cause of mortality in β-thalassemia [5].

Several studies have reported that the measurement of arterial intima−media thickness is a good

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indicator of subclinical atherosclerosis and a predictor of subsequent events that include myocardial infarction (MI) and stroke [6].

Proinflammatory cytokines and inflammation process play a key role in atherogenesis through leukocyte adhesion to endothelium and subendothelial migration, directly or indirectly by stimulating the expression of adhesion molecules such as soluble intercellular adhesion molecule-1 and soluble vascular cell adhesion molecule-1 on endothelial cells and by reducing endothelial-derived NO and its bioavailability [7].

Owing to the fact that elevation of asymmetrical dimethylarginine (ADMA) levels and inhibition of NO synthesis impair endothelial function and cause atherosclerosis, ADMA can be used as an early marker for endothelial dysfunction and an independent predictor of future cardiovascular events [8].

The aim of this study was to evaluate serum ADMA levels in pediatric patients who received a diagnosis of beta-thalassemia as a predictor of endothelial dysfunction.

2. Methods

After research Ethical Committee approval (ID:191219PEDI62), informed written consents were obtained from parents of all participants. This study was performed on 30 children diagnosed with β-thalassemia major (β-TM) (the patient group). There were 16 females and 14 males, who attended the hematology unit of the Paediatric Department, Menoufia University. The age range was between 1.5 and 17 years, and the mean age was 10.11 ± 4.36 years. The second group was 30 healthy children as a control group, which included 16 females and 14 males, with age ranging from 4 to 14 years and mean age of 11.23 ± 3 years. The study was performed in the period between January 2020 and December 2020. The exclusion criteria included the diagnosis of chronic diseases such as diabetes mellitus, hypertension, renal failure, hepatic disease, hypothyroidism/hyperthyroidism, coronary artery disease, and premature atherosclerosis.

The two groups were subjected to complete history taking with special consideration to onset age of thalassemia; first time and frequency of blood transfusion; blood transfusion index/year; and history of splenectomy. Complete clinical examination was performed and attention was paid to pallor, jaundice, and splenomegaly.

As for laboratory investigations, specimen collection and handling venous blood samples were collected for measurement of complete blood count using (ADVIA 2120) auto counter supplied by SIEMENS; liver and kidney function tests, which were performed using AU680 chemistry analyzer; and serum ADMA using SUN RED (ST) ELISA Kit from Shanghai (SHANGHAI SUNRED BIOLOGICAL TECHNOLOGY CO., LTD, Shanghai, China).

Data were analyzed using IBM SPSS Corp. Version 22.0 (SPSS Inc., Chicago, Illinois, USA). Qualitative data were described using number and percentage. Quantitative data were described using median and interquartile range for nonparametric data and mean and SD for parametric data after testing normality using Kolmogorov–Smirnov test. Significance of the obtained results was set at the 0.05 level. The statistical tests were χ^2-test, Monte–Carlo test, Student's *t*-test, and Mann–Whitney *U*-test.

We also performed the Spearman's rank-order correlation, receiver operating characteristic curve analysis, binary logistic regression, and linear regression analysis.

The sample sizing assumes the expected mean difference for serum levels of ADMA between β-thalassemia patients and controls to be 0.29 μmol/l [7]. To achieve 90% power and significant difference level of 2.5%, it was estimated that 20 participants per group were required. With a withdrawal/non-evaluable subject rate of 10%, 22 participants per group were recruited.

3. Results

There was no statistically significant differences between patients and controls regarding socio-demographic characteristics except for height and weight and BMI centiles. The most common clinical presentation in studied patients were pallor, jaundice, and splenomegaly. The most commonly used iron-chelating drug was oral deferasirox (Table 1).

Hemoglobin, hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and MCH concentration were statistically significant lower in the case group as compared with the control group. However, red cell distribution width, platelet count, white blood cell (WBC) count, serum ferritin, alanine transferase, and aspartate transferase were statistically significantly higher in the case group. Serum ADMA was statistically significant higher in the patient group. Mean carotid intima–media thickness (CIMT) values for right and left sides were statistically significant higher in the case group (Table 2). A significant positive correlation was found between serum ADMA and right CIMT (Table 3, Fig. 1).

Table 1. Sociodemographic characteristics and clinical data of the studied groups.

	Cases ($N = 30$)	Control ($N = 30$)	Test of significance	P value
Age (years)				
Mean ± SD	10.11 ± 4.36	11.23 ± 3.0	$t = 1.16$	0.25
Sex [N (%)]				
Male	16 (53.3)	16 (53.3)		
Female	14 (46.7)	14 (46.7)	$\chi^2 = 0$	1
Weight (kg)				
Mean ± SD	31.85 ± 13.04	31.30 ± 11.69	$t = 0.17$	0.87
Height (cm)				
Mean ± SD	132 ± 17.31	134.76 ± 26.19	$t = 0.46$	0.65
BMI (kg/m^2)				
Mean ± SD	16.52 ± 2.94	17.04 ± 2.56	$t = 0.73$	0.47
Weight centile [N (%)]				
<5	5 (16.7)	0		
Normal	25 (83.3)	30 (100)	$\chi^2 = 5.45$	0.02*
Height centile [N (%)]				
<5	6 (20)	0		
Normal	24 (80)	30 (100)	MC	0.005*
BMI centile [N (%)]				
<5	6 (20)	11 (36.7)		
Normal	24 (80)	19 (63.3)	MC	0.33
Clinical presentation [N (%)]				
Pallor		30 (100)		
Jaundice		30 (100)		
Splenomegaly		20 (66.7)		
Mean ± SD (cm)		9.8 ± 4.75		
Splenectomy		10 (33.3)		
Blood transfusion and chelation therapy				
Blood transfusion index (ml/kg/year)				
Median (IQR)		200 (130–300)		
Type of iron chelation		$n = 28$		
Deferasirox (Jadnue)		26 (92.9)		
Deferasirox (Exjad)		2 (7.1)		
Dose of chelator (mg/kg/day)				
Median (IQR)		15.5 (15.5–28)		

*Statistically significant ($P < 0.05$).
IQR, interquartile range; MC, Monte–Carlo test; t, Student's t-test.

Receiver operating characteristic analysis revealed the validity of ADMA in differentiating β-thalassemia cases from controls (Fig. 2).

4. Discussion

This study showed a statistically significant difference in the weight and height centiles between β-thalassemia cases and the healthy controls ($P = 0.02$ and 0.005, respectively). This was supported by Abdelsamei et al. [9], who reported a statistically significant difference regarding weight, as it ranged from 5th to 50th centile in cases versus 25th to 90th centile in controls, with P value of 0.025. The range of height was fifth to 90th centile compared with 25th–90th centile in controls, with P value of 0.001. As for BMI centiles, the range of cases was 3rd–97th, compared with 10th–97th centile in controls, with P value of 0.001.

In the current study, pallor and jaundice were reported in all the cases and splenomegaly was detected in 66.7%, whereas the remaining 33.3% of cases had splenectomy. Hemoglobin level, HCT value, MCV, MCH, and MCH concentration were statistically significantly lower in the case group as compared with the control group. As for red cell distribution width, platelet count, WBC count, serum ferritin, alanine transferase, and aspartate transferase were statistically significantly higher in the case group. In agreement, Hagag and colleagues showed that all children presented with pallor and jaundice and 90% with splenomegaly. In addition, the cases with β-thalassemia had significantly lower hemoglobin level, HCT value, MCV, and MCH. However, platelet count, WBC count, and serum ferritin were significantly increased in the case group [1].

Hershko [10] and Ghone et al. [11] demonstrated that iron overload in patients with thalassemia was the main outcome of multiple blood transfusions, which added about 100–200 ml of pure RBCs/kg/year (equivalent to 108–216 mg of Fe/kg/year). Increased iron absorption in patients with β-

Table 2. Comparison of laboratory and radiological investigations of the studied groups.

	Cases ($N = 30$)	Control ($N = 30$)	Test of significance	P value
Hb (g/dl)				
Mean ± SD	7.43 ± 1.37	11.22 ± 1.16	$t = 11.5$	<0.001*
HCT%				
Mean ± SD	25.40 ± 3.62	30.99 ± 2.22	$t = 7.21$	<0.001*
MCV (fl)				
Mean ± SD	60.87 ± 8.06	80.78 ± 4.07	$t = 6.35$	<0.001*
MCH (pg)				
Mean ± SD	24.54 ± 2.69	30.87 ± 2.66	$t = 9.15$	<0.001*
MCHC%				
Mean ± SD	30.45 ± 2.53	32.62 ± 1.53	$t = 4.0$	<0.001*
RDW%				
Median (IQR)	16.75 (14.25–18.6)	14.3 (12.9–15.9)	$z = 3.13$	0.002*
Platelet/mm^3				
Median (IQR)	428 (281.75–743.5)	260 (220.25–31.0)	$z = 6.66$	0.02*
WBCs/mm^3				
Median (IQR)	11.95 (7.03–48.70)	7.8 (5.85–10.0)	$z = 6.65$	<0.001*
Serum ferritin (ng/ml)				
Median (IQR)	2502 (1032.75–4607.75)	53.5 (65–80)	$z = 6.66$	<0.001*
ALT (Iμ/l)				
Median (IQR)	46.5 (25.75–87.75)	22.0 (19.0–30.0)	$z = 3.99$	<0.001*
AST (Iμ/l)				
Median (IQR)	47.5 (36.0–79.5)	20.0 (17.0–28.0)	$z = 6.13$	<0.001*
Serum urea (mg/dl)				
Mean ± SD	25.50 ± 9.01	25.53 ± 5.5	$t = 0.02$	0.99
Serum creatinine (mg/dl)				
Mean ± SD	0.62 ± 0.15	0.63 ± 0.12	$t = 0.38$	0.7
Serum asymmetric dimethyl argenine (ADMA)				
Serum ADMA (ng/l)				
Median (IQR)	23 958 (18 117.5–27 303.5)	2387 (1452–10 456.5)	$z = 6.28$	<0.001
Carotid intima–media thickness				
Right CIMT (mm)				
Mean ± SD	0.6 ± 0.013	0.4 ± 0.025	$t = 5.19$	0.012
Left CIMT (mm)				
Mean ± SD	0.6 ± 0.013	0.4 ± 0.012	$t = 5.2$	0.012

ADMA, asymmetrical dimethylarginine; ALT, alanine transferase; AST, aspartate transferase; CIMT, carotid intima–media thickness; IQR, interquartile range; MCH, mean corpuscular hemoglobin; MCHC, mean cell hemoglobin concentration; MCV, mean corpuscular volume; RDW, red blood cell distribution width; *t*, Student's *t*-test; *U*, Mann–Whitney *U*-test; WBCs, white blood cells.
*Statistically significant ($P < 0.05$).

thalassemia increases several folds than the normal daily intestinal iron absorption, that is, about 1–1.5 mg/day [12]. Iron overload is thought to be the main precipitating mechanism of immunological abnormalities; iron excess may derange the immune balance in favor of the growth of infectious organisms [13]. Iron overload also generates oxygen-free radicals and causes peroxidative tissue injury, leading to accelerated aging of immune system with subsequent gradual decline in responsiveness to antigens and abnormal T-cell functions [14].

Table 3. Correlation between serum ADMA and carotid intima–media thickness.

	Serum ADMA (ng/l)	
	*r**	*P*
Right CIMT	0.43	0.02*
Left CIMT	0.27	0.15

ADMA, asymmetrical dimethylarginine; CIMT, carotid intima–media thickness; *r*, Spearman correlation coefficient.
*Statistically significant.

In the current study, the best cutoff point of ADMA in differentiating cases of β-thalassemia was 10 458.5 ng/l with sensitivity of 96.7%, specificity of 76.7%, positive predictive value of 80.6%, negative predictive value of 95.8%, and total accuracy of 86.7%. Both Gursel et al. [6] and Mohamed et al. [7] agreed with our results regarding plasma ADMA levels, which were significantly higher in patients with β-thalassemia compared with healthy controls.

Despite the difference in the criteria of the included cases, Helmi et al. [8] showed that serum ADMA was significantly elevated in MI, thalassemia, and MI with thalassemia cases compared with the control group ($P < 0.01$, 0.001, and 0.01, respectively). ADMA supported MI diagnostic

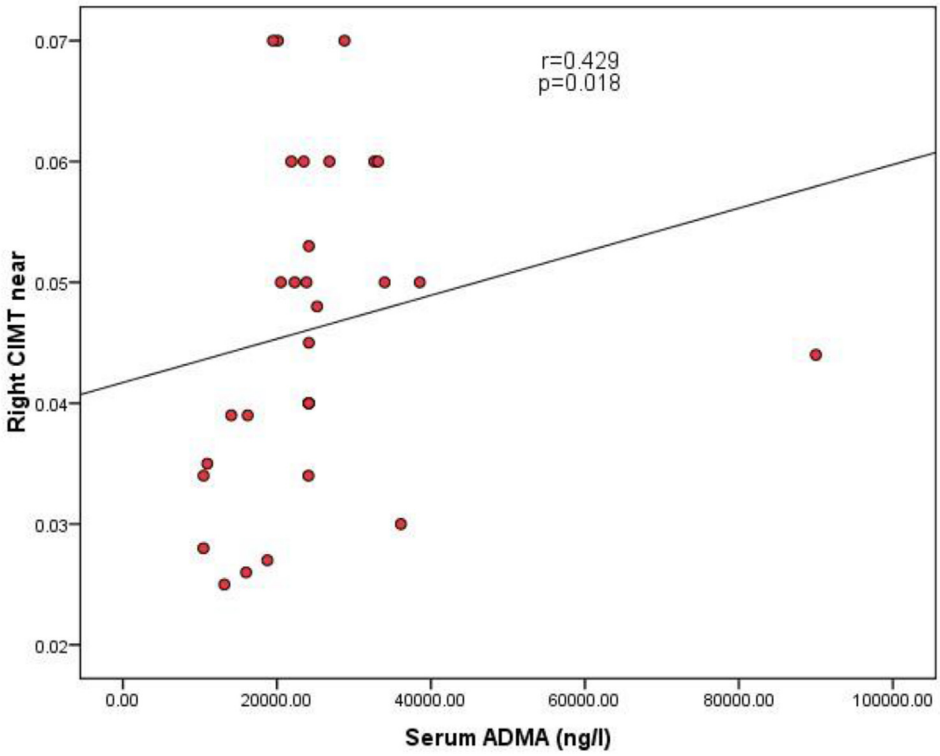

Fig. 1. Correlation between right carotid intima–media thickness (near) and serum asymmetric dimethylarginine among studied cases.

profile with an AUC of 0.85, sensitivity of 92.0%, and specificity of 91.9%.

Similarly, a report by D'Alecya and Billecke [15] suggested that hemolytic disorders might be associated with elevations in free plasma ADMA. Intact erythrocytes play an important role in storage of ADMA, and upon erythrocyte lysis, large amounts of free ADMA are generated by proteolysis of methylated proteins, which may affect plasma levels in hemolysis-associated diseases. This explains the increased levels of ADMA in patients with hemolysis [16].

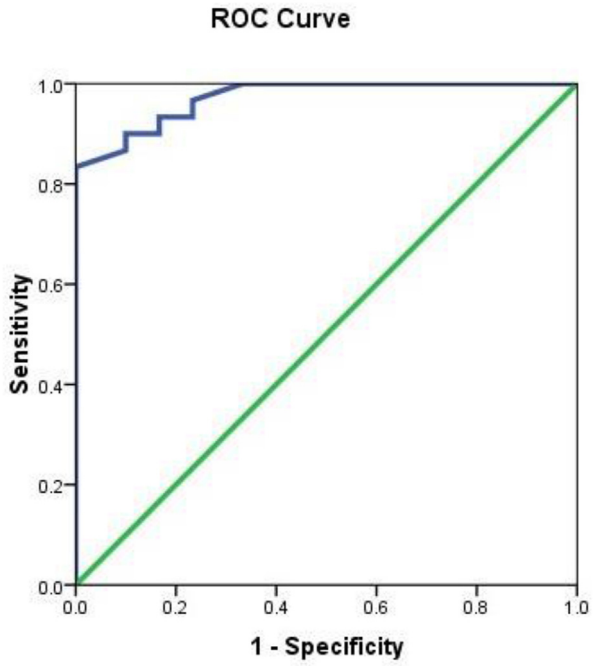

Fig. 2. Receiver operating characteristic curve of asymmetric dimethylarginine in differentiating cases of β-thalassemia major.

Another explanation for elevated ADMA levels could also be attributed to iron overload, which may reduce endothelium-derived NO bioactivity and increase ADMA levels [17]. In addition, the oxidative stress triggered by several cardiovascular risk factors interferes with the metabolism of ADMA following inhibition of dimethylarginine dimethylaminohydrolase that hydrolyzes asymmetric ADMA to dimethylamine and citrulline [18].

Abdelsamei et al. [9] showed that the CIMT ranged from 0.4 to 0.6 mm with a mean of 0.48 ± 0.02 mm in patients with thalassemia compared with 0.34–0.4 mm and a mean of 0.32 ± 0.05 mm in the controls. Cheung et al. [19] found an increase in the CIMT in patients with β-TM compared with controls (0.45 ± 0.04 vs. 0.39 ± 0.02 mm, $P < 0.001$). In addition, Adly et al. [5] reported that both right and left CIMT values were significantly increased in patients with thalassemia compared with controls (0.5 ± 0.14 vs.

0.31 ± 0.07 mm and 0.53 ± 0.1 vs. 0.32 ± 0.07 mm, respectively.

5. Conclusion

Our study confirms the previous literature findings that endothelial dysfunction is one of the main aspects implicated in β-TM, which eventually leads to cardiovascular complications, and that ADMA may be used an early diagnostic marker for endothelial dysfunction.

Conflict of interest

There are no conflicts of interest.

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