

The Effect of Plasma Endocan and Asymmetric Dimethyl Arginine Levels on Endothelial and Cardiac Functions in Children with Beta-Thalassemia Major

Beta-Talasemi Majörlü Çocuklarda Plazma Endocan ve Asimetrik Dimetil Arjinin Düzeylerinin Endotel ve Kardiyak Fonksiyonlara Etkisi

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Abstract

Introduction: Beta-thalassemia major (BTM) is an autosomal recessive disease characterized by hemolytic anemia. Increased iron load accumulating in the body as a result of frequent erythrocyte transfusions leads to endothelial dysfunction and cardiovascular complications. High asymmetric dimethylarginine (ADMA) levels disrupt endothelial function. Endocan is a soluble proteoglycan synthesized in vascular endothelial cells in many organs. High levels of endocan indicate endothelial dysfunction. We aimed to determine whether there is a correlation with cardiac evaluation instruments by evaluating circulating ADMA and endocan levels in BTM patient group and healthy control group and whether they can be a prognostic marker in terms of endothelial dysfunction and cardiovascular risk stratification.

Materials and Methods: A total of 39 patients with BTM and 39 age and gender-matched healthy children were enrolled in the study. Medical histories of all patients were recorded and physical examinations were performed. Complete blood count, reticulocyte count, serum ferritin and the iron level, liver, renal function tests, plasma ADMA and endocan, and proBNP. Cardiac examination results were evaluated on all children in both patient and control groups by a pediatric cardiologist.

Results: Mean ADMA level in the BTM group is higher than in the control group. Endocan levels in patients with BTM were not found to be statistically different. Serum endocan and ADMA levels were not associated with cardiovascular functions.

Conclusion: Although serum levels of ADMA were found to be significantly higher in BTM patients, high serum endocan and ADMA levels were not associated with cardiovascular functions.

Öz

Giriş: Beta-talasemi majör (BTM), hemolitik anemi ile karakterize otozomal resesif geçişli bir hastalıktır. Sık yapılan eritrosit transfüzyonları sonucu vücutta biriken artmış demir yükü, endotel disfonksiyonuna ve kardiyovasküler komplikasyonlara yol açar. Yüksek asimetrik dimetilarginin (ADMA) seviyeleri

Keywords

Anemia, cardiac function, child, thalassemia, endothelium

Anahtar kelimeler

Anemi, kalp fonksiyonu, çocuk, talasemi, endotel

Received/Geliş Tarihi : 24.09.2021

Accepted/Kabul Tarihi : 13.01.2022

DOI:10.4274/jcp.2022.88557

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endotel fonksiyon bozukluğunu gösteren bir belirteçtir. Endocan, birçok organdaki vasküler endotel hücrelerinde sentezlenen çözünür bir proteoglikandır. Yüksek endocan seviyeleri endotel disfonksiyonu gösterir. Bu çalışmada BTM hasta grubu ve sağlıklı kontrol grubunda dolaşımdaki ADMA ve endocan düzeylerini değerlendirerek kardiyovasküler fonksiyonlar ilişkisini; endotel disfonksiyonu ve kardiyovasküler risk sınıflaması açısından prognostik bir belirteç olup olmadığını belirlemeyi amaçladık.

Gereç ve Yöntem: Çalışmaya toplam 39 BTM'li hasta ile yaş ve cinsiyet açısından eşleştirilmiş 39 sağlıklı çocuk alındı. Tüm hastaların tıbbi öyküleri kaydedildi ve fizik muayeneleri yapıldı. Tam kan sayımı, retikülosit sayımı, serum ferritin ve demir düzeyi, karaciğer, böbrek fonksiyon testleri, plazma ADMA ve endocan ve proBNP tetkikleri değerlendirildi. Hem hasta hem de kontrol grubundaki tüm çocuklarda pediatrik kardiyolog tarafından kardiyak muayene sonuçları test edildi.

Bulgular: BTM grubunda ortalama ADMA düzeyi kontrol grubuna göre daha yüksektir. BTM'li hastalarda endocan düzeyleri istatistiksel olarak farklı bulunmadı. Serum endocan ve ADMA seviyeleri kardiyovasküler fonksiyonlarla ilişkisi gösterilemedi.

Sonuç: BTM hastalarında serum ADMA düzeyleri anlamlı olarak yüksek bulunmasına rağmen, yüksek serum endocan ve ADMA düzeyleri kardiyovasküler fonksiyonlarla ilişkili bulunmamıştır.

Introduction

BTM is an autosomal recessive disease characterized by hemolytic anemia in which one or more of the hemoglobin beta chain is produced defective (1). Regular erythrocyte transfusion is required to prevent clinical complications due to low hemoglobin levels (2). However, increased iron load accumulated in the body as a result of frequent erythrocyte transfusions leads to many organ damages, including endothelial dysfunction and cardiovascular complications (3,4). Peroxidative damage due to iron load caused by repeated blood transfusions and possibly coronary artery diseases are thought to be responsible for the pathogenesis (5,6). Vascular and cardiac complications are based on endothelial dysfunction and altered carotid artery structure, which are present in the nature of thalassemia (3).

It has been shown in many studies in the literature that the arginine-nitric oxide pathway triggered by hemolysis causes endothelial function (7,8). NO is a mediator that controls vascular tone by vasodilation, causes thrombocyte dysfunction, prevents the adhesion of leukocytes, and reduces vascular intimal cell production (9). NO is synthesized from the endothelium by converting L-arginine to L-citrulline by endothelial NO synthase (eNOS), whose activity is dependent on nicotinamide adenine dinucleotide phosphate (10). The addition of two methyl radicals to arginine by the action of methyltransferase nuclear proteins leads to the production of ADMA, which competes with L-arginine and reduces NO formation in the vascular wall (11). High ADMA levels inhibit NO synthesis. Thus, it disrupts the endothelial function and supports atherosclerosis. ADMA has been recognized as an early marker for endothelial

dysfunction and also as an independent factor for future cardiovascular disease (12,13).

Endocan [formerly known as endothelial cell-specific molecule-1, (ESM-1)] is a 50 kDa soluble proteoglycan that can be detected in circulation and is synthesized in vascular endothelial cells in many organs (14). It shows its effect by regulating the adhesion rate of cells to the vascular wall. High levels of endocan indicate endothelial dysfunction (15). Apart from that, it plays a role in cell migration, adhesion, proliferation and, neovascularization, which are biologically indispensable (16).

Considering this information, in our study, it was aimed to determine whether there is a correlation with cardiac evaluation instruments by evaluating circulating ADMA and endocan levels in transfusion-dependent BTM patient group and healthy control group. In addition, the utility of these markers in the assessment of endothelial dysfunction and cardiac risk stratification was investigated.

Materials and Methods

Ethics committee approval was obtained in accordance with the Helsinki declaration. A total of 39 patients with BTM and 39 age and gender-matched healthy children (control group) were enrolled in the study. Exclusion criteria were children with other chronic hemolytic anemia, cardiovascular diseases, congenital heart diseases, diabetes mellitus, hypertension, and previously known renal diseases. Patients with hepatic diseases were also excluded.

Medical histories of all patients were recorded and physical examinations were performed. Complete blood count, reticulocyte count, serum ferritin and iron level, liver, renal function tests, plasma ADMA and endocan, and proBNP values were evaluated in

one month period. Cardiac examination results were tested on all children in both patient and control groups by a pediatric cardiologist. Beta globin chain gene mutation analysis and treatment regimens of the patient group recorded. Circulating endocan and ADMA were determined with commercially available ELISA (enzyme-linked immunosorbent assay) kits (Wuhan USCN Business Co., Ltd., China.)

Echocardiographic investigations were performed in the Pediatric Cardiology Department of Konya Training and Research Hospital using General Electric Vivid S60 (General Electric Medical Systems, Horten, Norway) with 5.0 MHz transducers in our pediatric cardiology echocardiography laboratory by the same observer. Echocardiography including conventional Doppler, color images, and M-mode measurements was performed. Echocardiograms were recorded on a flash drive for repeated evaluation. All measurements were performed according to the American Society of Echocardiography. Patients with any congenital or acquired heart disease identified on echocardiography were excluded from the study group.

Ejection fraction and fractional shortening of the left ventricle (LV), interventricular septum diastolic diameter, left ventricular end-systolic and end-diastolic diameter (LVESD and LVEDD) and left ventricular posterior wall diastolic thickness were measured from 2-dimensional- guided M-mode echocardiographic tracings. End-diastolic and end-systolic volumes were also used to calculate EF by Simpson biplane method from the apical 4- and 2-chamber views. The pulse-wave Doppler echocardiographic parameters were as follows: early (E) and late (A) mitral diastolic velocities. Tissue Doppler velocities were obtained from 3 locations; the sample volume was positioned on the lateral aspect of each atrioventricular valve annulus and the basal portion of the interventricular septum. Peak early diastolic myocardial (e'), peak atrial systolic (a'), and peak systolic (s') myocardial velocities were measured using this technique.

Statistical Analysis

Categorical variables were expressed as numbers and percentages, whereas continuous variables were summarized as mean and standard deviation. Chi-square test was used to compare categorical variables between the groups. The normality of distribution for continuous variables was confirmed with the Shapir-

Wilk test. For comparison of continuous variables between groups, the Student's t-test or Mann-Whitney U test was used depending on whether the statistical hypotheses were fulfilled or not. To evaluate the correlations between measurements, Pearson-correlation coefficient or Spearman-rank correlation coefficient was used depending on whether the statistical hypotheses were fulfilled or not. All analyses were performed using IBM SPSS Statistics Version 20.0 statistical software package. The statistical level of significance for all tests was considered to be 0.05.

Results

There was no statistically significant difference between the study and control groups in terms of age and gender. Table 1, 2, Figure 1 show the demographic, laboratory characteristics, and mutation distribution of patients with BTM and the control group. According to the comparisons, a statistically significant difference was found between the two groups in terms of ADMA averages. Accordingly, the mean ADMA in the BTM group is higher than in the control group. When the groups were compared in terms of laboratory measurements and metabolic properties, proBNP, hemoglobin (hgb), hematocrit (htc), mean corpuscular volume (MCV), red blood cell (RBC), reticulocyte, ferritin, and iron measurements were found to be different between the groups. While hgb, htc, MCV and RBC were lower in the BTM, other measurements were higher than in the control group.

ADMA and laboratory measurements were examined in the patient group, there was no correlation between ADMA and other parameters (Table 2, 4).

Endocan and laboratory measurements were analyzed in the patient group, there was no correlation between endocan and other parameters (Table 3).

When the echocardiological measurements of the groups were compared, differences were found between the groups in LVISD, LVIDs (left ventricular end-diastole), LVPWd (left ventricular posterior wall), LPWs (left posterior wall), ejection fraction (EF), mitral E, A mitral, S mitral, S septal, tricuspid A and TR measurements. LVPWd, LPWs, EF, and S mitral measurements were lower in beta-thalassemia group, while other measurements were higher (Table 4).

Among the groups, ADMA and endocan and echocardiography parameters were studied within the groups, only a weak negative correlation was found

Mutation	n (%)
c.118C>T (homozygous)	11
IVS-I-110 G>A (C.93-21 G>A) (homozygous)	7
IVS-I-5 G>C (c.92+5 G>C) (heterozygous) CODON 39 C>T (c.118C>T p.Gln140Ter) (heterozygous)	6
IVS 1.110 [G>A] (double heterozygous)	2
IVS-I-6 T>C (heterozygous) COD 39>T (heterozygous)	2
IVS-I-130 G>C (c.93-1 G>C) (heterozygous) IVS-II-1 G>A (c.315+1 G>A) (heterozygous)	2
IVS-I-1 G>A (C.92+1 G>A) (homozygous)	2
IVS-I-6 T>C (c.92+6 T>C) (heterozygous) c.135delC (p.F46Lfs*16)the portuguese type beta+ (heterozygous)	1
c.112delT (p.Trp38GlyfsTer24) (W38Gfs*24) (homozygous)	1
c.17_18delCT (p.Pro6ArgfsTer17)(P6Rfs*17) (homozygous)	1
HBB: Codon 5 (-CT) c.17_18delCT) (homozygous)	1
IVS1-1G>C (c.93-1G>C) (homozygous)	1
rsa 11p15.4 (HBB) x0 Turkish 7.6 kb B-Thal (double deletion) (HGMD ID: CG931306)	1
IVS-II-1 G>A (c.315+1 G>A) (homozygous)	1

Table 2. Demographic and laboratory characteristics of patients with beta-thalassemia major and the control group

Variables	Study group (n=39)	Control group (n=39)	p-value
Female/male	19 (49%) 20 (51%)	16 (41%) 23 (59%)	0.495
Age (months)	82.2±59.1	81.23±58.1	0.945
Hgb (g/dL)	6.31±1.12	13.0±0.7	<0.001
Htc (%)	19.2±3.7	38.3±2.3	<0.001
MCV (fL)	67.4±10.5	87.1±8.9	<0.001
RBC (10 ⁶ /μL)	2.68±0.84	4.6±0.41	<0.001
Reticulocyte (%)	2.98±1.25	1.17±0.23	<0.001
Iron (mcg/dL)	132.6±58.8	99.1±33.0	0.004
ALT (IU/L)	29.3±27.3	27.1±16.4	0.952
AST (IU/L)	41.5±24.4	33.2±14.9	0.073
Ferritin (ng/mL)	1394.7±1389.6	103.3±41.1	<0.001
Pro-BNP (pg/mL)	100.4±64.2	53.5±2.8	0.013
ADMA (ng/mL)	679.7±171.9	455.3±258.7	<0.001
Endocan (pg/mL)	337.5±344.0	218.14±171.1	0.057

Hgb: Hemoglobin, MCV: Mean corpuscular volume, RBC: Red blood cell, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, pro-BNP: Pro-brain natriuretic peptide, ADMA: Asymmetric dimethylarginine

between ADMA and tricuspid A. Tricuspid A decreases as the serum level of ADMA increases (Table 5).

Discussion

There are many complications related to the disease in children with BTM. Cardiac complications are the most important of these complications that increase mortality and morbidity (17). Studies have suggested that the most common cardiac complications are heart failure and arrhythmias (18). It has been proven in many studies that endothelial dysfunction plays an important role in the pathogenesis of cardiovascular diseases (19,20). In this study, we aimed to investigate the effects of ADMA and endocan, which are responsible for endothelial dysfunction in many non-hematological cardiac diseases, on cardiovascular function in children with transfusion-dependent BTM.

ADMA is a modified amino acid found in blood and shows its effect by blocking the synthesis of NO. In the control of vascular tone, NO plays a crucial role. As a result, it makes a significant contribution to atherosclerosis (21). In this study, we conducted 39 pediatric patients diagnosed with BTM and gender/age-

similar 39 healthy children, and we observed that there was a significant difference between serum ADMA levels ($p < 0.001$). There are some studies similar to our results showing elevations in plasma ADMA levels in BTM patients. In the study performed by Gürsel et al. (5) with 31 children diagnosed with BTM and 36 healthy controls, by comparing serum soluble vascular cell adhesion molecule-1 (sVCAM-1), soluble intercellular adhesion molecule-1 (sICAM-1), P-selectin, and pentraxin-3 levels, a significant relationship was found between ADMA levels and endothelial dysfunction. In another study conducted with 36 BTM patients and healthy controls in which echocardiographic

features were compared, it was found that ADMA levels were statistically significantly higher in the patient group. This result may be interpreted as the increase in serum ADMA levels in BTM patients is an indicator of endothelial dysfunction. In our study, the hemolytic rate was not found to have a positive correlation with serum ADMA levels (22). This result, which contradicts with the literature, may be due to the fact that the average age of our patient group is lower than the studies mentioned above (82.2 ± 59.1 mo. vs 12 and 11.25 yrs) and that hemolytic disease has not yet caused significant endothelial dysfunction. However, in our study, it was observed that there was no correlation between ADMA and parameters related to disease characteristics in the BTM group (Table 1).

The basic diagnostic parameters of BTM (low levels of Hb, Htc, MCV, RBC, reticulocyte, iron, and elevated serum ferritin) were found to be significantly different in the patient group as expected. In addition, it was noticed that the proBNP ($p = 0.013$) values were statistically significantly higher in the BTM group (Table 1). Although the proBNP values were significantly higher in the patient group with BTM, it was observed that they did not show a significant correlation with ADMA. In the BTM patient group, cardiac EF was found to be significantly lower than the control group (Table 3). Cardiac dysfunction due to iron load is an expected complication, and many reports have been published showing that cardiac EF is low in BTM patients (23,24).

Endocan is a soluble proteoglycan released by vascular endothelial cells, which has been linked to the growth of vascular tissue. It is widely known that endothelial inflammation is crucial in cardiovascular disease pathophysiology (25). The relationship of endocan with endothelial function and inflammation has been well defined in previous studies on many diseases such as cancers, systemic inflammatory diseases, and cardiovascular diseases (26). Since it is an indicator of vascular endothelial dysfunction, its role in cardiovascular diseases has attracted the attention of many authors and has been proven by many studies on the subject (15,27,28). However, the aforementioned studies were carried out on adults, and the levels in BTM patients, which are well known to disrupt the endothelial integrity of the endocan, and its relationship with the cardiovascular system have not been discussed before. It is well known that iron

Table 3. Correlations in beta thalassemia group

Pairs	Correlation coefficient	p
ADMA vs		
Age (months)	0.120	-0.253
Endocan	0.057	0.308
proBNP (pg/mL)	0.817	-0.038
Hgb (g/dL)	0.077	0.286
Htc (%)	0.144	0.238
MCV (fL)	0.623	-0.081
RBC ($10^6/\mu\text{L}$)	0.513	0.108
Retikülosit (%)	0.056	-0.429
Ferritin (ng/mL)	0.112	-0.258
Iron (mcg/dL)	0.928	0.015
ALT (IU/L)	0.326	-0.161
AST (IU/L)	0.459	-0.122
Pairs	Correlation coefficient	p
Endocan vs		
Age (month)	0.888	-0.023
proBNP (pg/mL)	0.761	0.050
Hgb (g/dL)	0.347	0.155
Htc (%)	0.474	0.118
MCV (fL)	0.960	0.008
RBC ($10^6/\mu\text{L}$)	0.523	0.105
Retikülosit (%)	0.415	-0.134
Ferritin (ng/mL)	0.455	0.123
Iron (mcg/dL)	0.797	0.044
ALT (IU/L)	0.629	0.080
AST (IU/L)	0.491	-0.114

Hgb: Hemoglobin, MCV: Mean corpuscular volume, RBC: Red-blood cell, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, pro-BNP: Pro-brain natriuretic peptide, ADMA: Asymmetric dimethylarginine

overload causes toxicity in many organs, including the cardiovascular system, in β -thalassemia patients. Cardiac mortality and morbidity remain high in this population, despite the fact that iron chelation therapy has enhanced prognosis. Endothelial cell activation is well documented, and vascular complications are more common in BTM (29). In our study, serum endocan levels in patients with BTM were not found to be statistical difference (337.5 ± 344.0 pg/mL vs 218.14 ± 171.1 pg/mL) ($p=0.057$) (Table 1). To the best of our knowledge, there are no similar studies in the literature comparing serum endocan levels of healthy children with beta-thalassemia major patients. It has been shown that the release of endocan from the endothelium is minimal under physiological conditions and increases in conditions that lead to activation such as endothelial disorders (30). In addition, when

endocan and echocardiographic parameters were compared, no statistically significant relationship was found between them (Table 4, 5). The low average age of the BTM patient group may have contributed to this result.

When the relationship between ADMA and endocan levels and 16 BTM patients who had cardiac T2* MR was evaluated, it was observed that there was no correlation ($r=0.157$, $p=0.560$ and $r=-0.133$ ve $p=0.625$ respectively) To our knowledge, there is no study comparing serum ADMA/endocan levels with cardiac T2* MR in literature. Both the low number of BTM patients with cardiac T2* MRI and the wide distribution of measurements may affect this result (31). The relationship between ADMA and Endocan with echocardiography parameters was evaluated. However, a significant correlation that would affect

Table 4. The comparison of echocardiological measurements between the patient and control group

Group	Control (mm)	Dimensions	p
Beta-thalassemia major (mm)			
IVSd-mean \pm SD	5.68 \pm 1.11	5.9 \pm 1.23	0.412
IVSs-mean \pm SD	9.08 \pm 3.44	9.03 \pm 1.84	0.930
LVIDd-mean \pm SD	35.4 \pm 7.6	5.62 \pm 1.21	<0.001
LVIDs-mean \pm SD	22.9 \pm 6.0	9.1 \pm 2.01	<0.001
LVPWd-mean \pm SD	5.54 \pm 1.14	37.0 \pm 8.5	<0.001
LPWs-mean \pm SD	10.1 \pm 10.4	23.5 \pm 5.4	<0.001
EF-mean \pm SD	64.4 \pm 6.9	67.4 \pm 5.7	0.045
FS-mean \pm SD	34.5 \pm 5.4	35.6 \pm 6.0	0.416
LA-mean \pm SD	21.3 \pm 5.7	20.4 \pm 4.2	0.441
Ao-mean \pm SD	19.6 \pm 8.2	18.5 \pm 4.3	0.494
Simpsons EF-mean \pm SD	60.0 \pm 9.4	62.1 \pm 3.6	0.196
Mitral E-mean \pm SD	121.9 \pm 17.1	106.3 \pm 14.2	<0.001
Mitral A-mean \pm SD	79.7 \pm 19.0	72.9 \pm 12.8	0.069
E mitral-mean \pm SD	16.9 \pm 4.4	16.0 \pm 2.7	0.293
A mitral-mean \pm SD	8.65 \pm 2.45	7.13 \pm 1.79	0.003
S mitral-mean \pm SD	9.27 \pm 2.06	10.56 \pm 2.06	0.008
E septal-mean \pm SD	14.7 \pm 2.6	14.6 \pm 1.6	0.821
S septal-mean \pm SD	8.97 \pm 1.71	7.97 \pm 1.99	0.022
A septal-mean \pm SD	9.19 \pm 3.01	10.33 \pm 3.72	0.146
Tricuspid E-mean \pm SD	19.1 \pm 3.7	17.8 \pm 3.2	0.124
Tricuspid A-mean \pm SD	12.1 \pm 3.0	10.4 \pm 2.2	0.006
Tricuspid S-mean \pm SD	13.6 \pm 2.8	13.7 \pm 2.0	0.899

LPWD: Left ventricular posterior wall dimension, IVSd: Interventricular septum diastolic dimension, IVSs: Interventricular septum systolic dimension, LVIDd: Left ventricular end-diastolic dimension, LVIDs: Left ventricular end-systolic dimension, EF: Ejection fraction, FS: Shortening fraction, LA: Left atrium, Ao: Aort, E: Early, A: Late, s: Systolic, d: Diastolic, SD: Standard deviation

Table 5. Comparison of ADMA, endocan and echocardiography parameters of patient and control groups				
Pairs	Beta-thalassemia major	Control	Correlation coefficient (p)	Correlation coefficient (p)
ADMA vs				
IVSd	0,363	-0.154	0.957	0.009
IVSs	0.719	-0.061	0.402	-0.138
LVIDd	0.131	-0.253	0.244	-0.191
LVIDs	0.116	-0.263	0.771	-0.048
LVPWd	0.216	-0.208	0.672	0.070
LPWs	0.882	0.025	0.796	0.043
EF	0.584	0.093	0.853	-0.031
FS	0.779	0.048	0.925	0.016
LA	0.074	-0.297	0.995	-0.001
Ao	0.693	-0.067	0.567	-0.095
Simpsons EF	0.348	0.159	0.548	-0.099
Mitral E	0.902	-0.021	0.346	0.155
Mitral A	0.599	-0.089	0.754	0.052
E mitral	0.762	-0.052	0.389	0.142
A mitral	0.430	-0.134	0.678	-0.069
S mitral	0.148	0.242	0.214	0.203
E septal	0.371	0.151	0.808	-0.040
S septal	0.383	0.148	0.916	-0.018
A septal	0.877	0.026	0.951	0.010
Triküspit E	0.228	-0.203	0.744	0.054
Triküspit A	0.017	-0.390	0.856	0.030
Triküspit S	0.297	0.176	0.336	0.158
TAPSE	0.421	-0.136	0.329	0.160
TY	0.205	-0.216	0.896	0.022
Pairs	Beta-thalassemia major	Control	Correlation coefficient (p)	Correlation coefficient (p)
Endocan vs				
IVSd	0.210	-0.211	0.744	-0.054
IVSs	0.256	-0.192	0.298	0.171
LVIDd	0.384	0.147	0.583	-0.091
LVIDs	0.108	0.269	0.639	0.078
LVPWd	0.162	-0.235	0.496	0.112
LPWs	0.544	-0.103	0.765	0.049
EF	0.375	-0.150	0.759	0.051
FS	0.581	-0.094	0.668	0.071
LA	0.410	0.140	0.648	0.075
Ao	0.366	0.153	0.666	-0.071
Simpsons EF	0.349	-0.158	0.931	-0.014
Mitral E	0.252	0.193	0.668	-0.071
Mitral A	0.409	0.140	0.497	-0.112

Table 5. Continued				
Pairs	Beta-thalassemia major	Control	Correlation coefficient (p)	Correlation coefficient (p)
Endocan vs				
E mitral	0.906	0.020	0.163	0.228
A mitral	0.535	-0.105	0.327	-0.161
S mitral	0.644	0.078	0.797	0.043
E septal	0.419	0.137	0.741	-0.055
S septal	0.347	-0.159	0.669	-0.071
A septal	0.847	-0.033	0.984	0.003
Tricuspid E	0.431	-0.134	0.229	-0.197
Tricuspid A	0.388	-0.146	0.525	0.105
Tricuspid S	0.687	0.068	0.966	-0.007
TAPSE	0.772	0.049	0.939	-0.013
TY	0.734	-0.059	0.421	0.132

TAPSE: Tricuspid annular plane systolic excursion, LPWD: Left ventricular posterior wall dimension, IVSd: Interventricular septum diastolic dimension, IVSs: Interventricular septum systolic dimension, LVIDd: Left ventricular end-diastolic dimension, LVIDs: Left ventricular end-systolic dimension, EF: Ejection fraction, FS: Shortening fraction, LA: Left atrium, Ao: Aort, E: Early, A: Late, s: Systolic, d: Diastolic

cardiac functions could not be established except tricuspid a. Interestingly, in the study conducted by Mohamed et al. (22) with 30 BTM and healthy children, tricuspid regurgitant jet velocity was investigated and it was revealed that high ADMA levels in BTM patients could be associated with pulmonary hypertension. In our study, there was no significant increase in pulmonary artery pressure in BTM patients.

Soluble vascular cell adhesion molecule (sVCAM-1) and soluble intercellular adhesion molecule (sICAM-1) are among the most studied agents in endothelial dysfunction indicator studies in BTM patients. Serum levels of plasma sVCAM-1 and sICAM-1 molecules have been shown to increase inflammation and endothelial dysfunction (29). However, in a thesis study evaluating endothelial

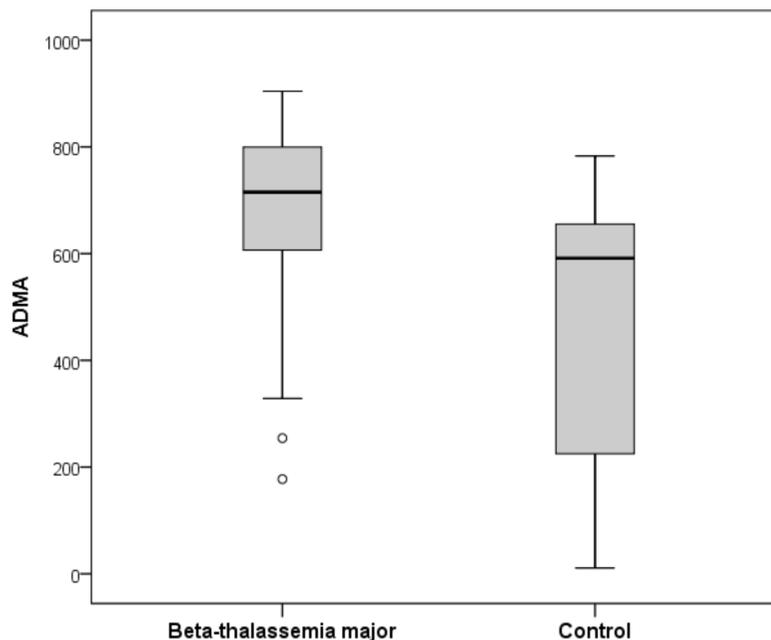


Figure 1. The comparison of serum ADMA levels between the patient and control group. When the correlations between ADMA and laboratory measurements were examined in the patient group, there was no correlation between ADMA and other parameters.

and cardiac functions using endothelial activation indicators in BTM patients, it was shown that sVCAM-1, sICAM-1, neopterin, and endothelin-1 did not differ in patients with and without cardiac hypertrophy (32).

The strength of our study is that it is the first study in which endocan was studied in patients with BTM and its relationship with cardiac functions was investigated. The weakness of the study is that our study was conducted with a relatively small number of patients and the inclusion of patients with low mean age, who were still partially affected by BTM.

Conclusion

ADMA and endocan are new inflammatory markers found in systemic inflammatory and cardiovascular diseases that could be used to predict endothelial damage. Although serum levels of ADMA were found to be significantly higher in BTM patients, serum endocan and ADMA levels were not associated with cardiovascular functions. However, for a more meaningful result, it is necessary to study with more patients with higher average age.

Ethics

Ethics Committee Approval: Ethics committee approval was obtained in accordance with the Helsinki declaration.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

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