# The Effect of plasma endocan and asymmetric dimethyl arginine levels on endothelial and cardiac functions in children with beta thalassemia major

Utku Aygüneş[1], Ümmügülsüm Can [2], Melih Doğan [2], Merter Keçeli[2], and Hatice Koçak Eker[2]

[1]Konya Training and Research Hospital
[2]Konya Training and Research Hospital

April 05, 2024

**Abstract**

Background: Beta-thalassemia major (BTM) is an autosomal recessive disease characterized by hemolytic anemia. Increased iron load accumulated in the body as a result of frequent erythrocyte transfusions leads to endothelial dysfunction and cardiovascular complications. High asymmetric dimethylarginine (ADMA) levels disrupts 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. 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, reticulocytic count, serum ferritin and iron level, liver, renal function tests, plasma ADMA and endocan and proBNP. Cardiac examination results by a pediatric cardiologist were tested on all children in both patient and control groups. Results: Mean ADMA in the BTM group is higher than in the control group. 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). Serum endocan and ADMA levels were not associated with cardiovascular functions. Conclusions: Although serum levels of endocan were found to be significantly higher in BTM patients, high serum endocan and ADMA levels were not associated with cardiovascular functions.

## 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 damage, including endothelial dysfunction and cardiovascular complications.[3,4] Peroxidative damage due to iron load caused by repeated blood transfusions and possible 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 and whether they can be a prognostic marker in terms of endothelial dysfunction and cardiovascular risk stratification.

## MATERIAL 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, reticulocytic count, serum ferritin and iron level, liver, renal function tests, plasma ADMA and endocan and proBNP. Cardiac examination results by a pediatric cardiologist were tested on all children in both patient and control groups. Beta globin chain gene mutation analysis and treatment regimens of 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. A full 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 vol- ume was positioned on the lateral aspect of each atrioventricular valve annulus and on the basal portion of interventricular septum. Peak early diastolic myocardial (e'), peak atrial systolic (a'), and peak systolic (s') myocardial velocities were measured using this technique.

Patients older than 8 years were also evaluated with cardiac T2 * MR imaging in Department of Radiology by a pediatric radiologist. All MR scans were performed on a 1.5 T Magnetom Aera MR scanner (Siemens, Germany) using a 4-channel anterior phased array coilat single center. A transverse slice through the center of the liver was imaged using a multi-echo single breath-hold gradient echo T2* sequence with a range of

echo times (TE 0.90-15.0 ms). Analysis was performed on a computer using Thalassemia - Tools software (a CMRtools plug-in; Cardiovascular Imaging Solutions, London, UK). Patients with a cardiac T2 * value of 15-20 ms and mild iron overload were grouped as mild, those with 8-14 ms as moderate, and those with <8 ms as severe.

**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 Shapiro 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.

SPSS referansı : IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.

## RESULTS

Table 1, 2, figure 1 and 2 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 3, 4).

Endocan and laboratory measurements were analyzed in the patient group, there was no correlation between endocan and other parameters (Table 4, figure 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 5, figure 3).

Among the groups, ADMA and endocan and echocardiography parameters were studied within the groups, only a weak negative correlation was found between ADMA and tricuspid A. Tricuspid A decreases as the serum level of ADMA increases (Table 5, figure 4).

## 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 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. 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.[5] 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. The hemolytic rate was 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 2).

The basic diagnostic parameters of BTM (low levels of Hb, Htc, MCV, RBC, reticulosit, iron and elevated serum ferritin) were found to be significantly different in the patient group as expected. In addition, it was noticed that the ESR ($p<0,001$) and proBNP ($p=0,013$) values were statistically significantly higher in the BTM group (Table 2). Although the proBNP and ESR 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 higher than the control group (Table 4). 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.[14] Endothelial inflammation is crucial in cardiovascular disease physiopathology[25]. The relationship of endocan with endothelial function and inflammation has been well defined in previous studies on many diseases suc 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 overload causes toxicity in many organs, including the cardiovascular system, in b-thalassemic 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 2). 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 increase 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 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 have an effect on this result.[31]The relationship between ADMA and Endocan with echocardiography parameters was evaluated. However, a significant correlation that would affect cardiac

functions could not be established except tricuspid a. Interestingly, in the study conducted by Mohamed et al 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.[22] 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 in inflammation and endothelial dysfunction.[29] However, in a thesis study evaluating endothelial 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 a low mean age, who were still partially affected by BTM.

In 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 a higher average age.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## REFERENCES

1. Origa R. β-Thalassemia. *Genet Med* . 2017;19(6):609-619. doi:10.1038/gim.2016.173

2. Kwiatkowski JL. Hemoglobinopathies. In: Lanzkowsky P, Lipton JM FJ, ed. *Lanzkowsky's Manual of Pediatric Hematology and Oncology.* 6th ed. Elsevier; 2016:188.

3. Stoyanova E, Trudel M, Felfly H et al. Vascular Endothelial Dysfunction in b-Thalassemia Occurs Despite Increased eNOS Expression and Preserved Vascular Smooth Muscle Cell Reactivity to NO. *PLoS One* . 2012;7(6):38089. doi:10.1371/journal.pone.0038089

4. Paul A, Thomson VS, Refat M et al. Cardiac involvement in beta-thalassaemia: current treatment strategies. *Postgrad Med* . 2019;131(4):261-267. doi:10.1080/00325481.2019.1608071

5. Gursel O, Tapan S, Sertoglu E, et al. Elevated plasma asymmetric dimethylarginine levels in children with beta-thalassemia major may be an early marker for endothelial dysfunction. *Hematology* . 2018;23(5):304-308. doi:10.1080/10245332.2017.1396027

6. Cusmà Piccione M, Piraino B, Zito C, et al. Early identification of cardiovascular involvement in patients with β-thalassemia major.*Am J Cardiol* . 2013;112(8):1246-1251. doi:10.1016/j.amjcard.2013.05.080

7. Frimat M, Boudhabhay I, Roumenina LT. toxins Hemolysis Derived Products Toxicity and Endothelium: Model of the Second Hit. Published online 2019. doi:10.3390/toxins11110660

8. Morris C, Gladwin M, Kato G. Nitric Oxide and Arginine Dysregulation: A Novel Pathway to Pulmonary Hypertension in Hemolytic Disorders.*Curr Mol Med* . 2008;8(7):620-632. doi:10.2174/156652408786241447

9. Sandoo A, Veldhuijzen van Zanten JJC., Metsios GS et al. The Endothelium and Its Role in Regulating Vascular Tone. *Open Cardiovasc Med J* . 2015;4(1):302-312. doi:10.2174/1874192401004010302

10. Gambardella J, Khondkar W, Morelli MB et al. Arginine and endothelial function. *Biomedicines* . 2020;8(8). doi:10.3390/BIOMEDICINES8080277

11. Landim MBP, Casella Filho A, Chagas ACP. Asymmetric dimethylarginine (ADMA) and endothelial dysfunction: Implications for atherogenesis. *Clinics* . 2009;64(5):471-478. doi:10.1590/S1807-59322009000500015

12. Böger RH, Maas R, Schulze F et al. Asymmetric dimethylarginine (ADMA) as a prospective marker of cardiovascular disease and mortality-An update on patient populations with a wide range of cardiovascular risk. *Pharmacol Res* . 2009;60(6):481-487. doi:10.1016/j.phrs.2009.07.001

13. Dogru T, Genc H, Tapan S, et al. Elevated asymmetric dimethylarginine in plasma: An early marker for endothelial dysfunction in non-alcoholic fatty liver disease? *Diabetes Res Clin Pract* . 2012;96(1):47-52. doi:10.1016/j.diabres.2011.11.022

14. Sarrazin S, Adam E, Lyon M, et al. Endocan or endothelial cell specific molecule-1 (ESM-1): A potential novel endothelial cell marker and a new target for cancer therapy. *Biochim Biophys Acta - Rev Cancer* . 2006;1765(1):25-37. doi:10.1016/j.bbcan.2005.08.004

15. Balta S, Mikhailidis DP, Demirkol S et al. Endocan: A novel inflammatory indicator in cardiovascular disease? *Atherosclerosis* . 2015;243(1):339-343. doi:10.1016/j.atherosclerosis.2015.09.030

16. Delehedde M, Devenyns L, Maurage CA et al. Endocan in cancers: A lesson from a circulating dermatan sulfate proteoglycan. *Int J Cell Biol* . Published online 2013. doi:10.1155/2013/705027

17. Cardiac Complications in Thalassaemia Major - Guidelines for the Management of Transfusion Dependent Thalassaemia (TDT) - NCBI Bookshelf. Accessed March 11, 2021. https://www.ncbi.nlm.nih.gov/books/NBK269371/

18. Koohi F, Kazemi T, Miri-Moghaddam E. Cardiac complications and iron overload in beta thalassemia major patients—a systematic review and meta-analysis. *Ann Hematol* . 2019;98(6):1323-1331. doi:10.1007/s00277-019-03618-w

19. Biswas I, Khan GA. Endothelial Dysfunction in Cardiovascular Diseases. *Endothel Dysfunct Cardiovasc Dis* . 2014;2014(3):291-308. www.intechopen.com

20. Haybar H, Shahrabi S, Rezaeeyan H et al. Endothelial Cells: From Dysfunction Mechanism to Pharmacological Effect in Cardiovascular Disease. *Cardiovasc Toxicol* . 2019;19(1):13-22. doi:10.1007/s12012-018-9493-8

21. Dowsett L, Higgins E, Alanazi S et al. ADMA: A Key Player in the Relationship between Vascular Dysfunction and Inflammation in Atherosclerosis. *J Clin Med* . 2020;9(9):3026. doi:10.3390/jcm9093026

22. Mohamed ES, Ibrahim B, Amr D et al. Asymmetric dimethylarginine levels in children with β-thalassemia and their correlations to tricuspid regurgitant jet velocity. *Pediatr Blood Cancer* . 2014;61(9):1540-1543. doi:10.1002/pbc.25076

23. Chouliaras GL, Kattamis A, Berdoukas V et al. Cardiac magnetic resonance in transfusion dependent thalassaemia: Assessment of iron load and relationship to left ventricular ejection fraction. *Br J Haematol* . 2010;151(4):397-401. doi:10.1111/j.1365-2141.2010.08365.x

24. Davis BA, O'Sullivan C, Jarritt PH et al. Value of sequential monitoring of left ventricular ejection fraction in the management of thalassemia major. *Blood* . 2004;104(1):263-269. doi:10.1182/blood-2003-08-2841

25. Unlu M, Karaman M, Ay SA, et al. The comparative effects of valsartan and amlodipine on vascular microinflammation in newly diagnosed hypertensive patients. *Clin Exp Hypertens* . 2013;35(6):418-423. doi:10.3109/10641963.2012.739237

26. Kali A, Rathan Shetty KS. Endocan: A novel circulating proteoglycan. *Indian J Pharmacol* . 2014;46(6):579-583. doi:10.4103/0253-7613.144891

27. Zhao T, Kecheng Y, Zhao X, et al. The higher serum endocan levels may be a risk factor for the onset of cardiovascular disease: A meta-analysis. *Med (United States)* . 2018;97(49). doi:10.1097/MD.0000000000013407

28. Bessa J, Albino-Teixeira A, Reina-Couto M et al. Endocan: A novel biomarker for risk stratification, prognosis and therapeutic monitoring in human cardiovascular and renal diseases. *Clin Chim Acta* . 2020;509:310-335. doi:10.1016/j.cca.2020.07.041

29. Aggeli C, Antoniades C, Cosma C, et al. Endothelial dysfunction and inflammatory process in transfusion-dependent patients with beta-thalassemia major. *Int J Cardiol* . 2005;105(1):80-84. doi:10.1016/j.ijcard.2004.12.025

30. Menon P, Kocher O, Aird W. Abstract 15455: Endothelial Cell Specific Molecule-1 (ESM-1), a Novel Secreted Proteoglycan Stimulates Vascular Smooth Muscle Cell Proliferation and Migration. *undefined* . Published online 2011.

31. Daar S, Al Khabori M, Al Rahbi S et al. Cardiac T2* MR in patients with thalassemia major: a 10-year long-term follow-up. *Ann Hematol* . 2020;99(9):2009-2017. doi:10.1007/s00277-020-04117-z

32. Unsal C. Cardiac Functions and Indicators Of Endothelial Activation in Patients With B-Thalassemia Major. Published online 2005.

**LEGENDS**

**TABLE 1** Mutation distribution rates of BTM patients.

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

**TABLE 3** Correlations in beta-thalassemia patient group.

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

**TABLE 5** Comparison of ADMA, endocan and echocardiography parameters of patient and control groups.

**FIGURE 1** Comparison of laboratory parameters between the patient and control group.

**FIGURE 2** Comparison of serum ADMA levels between the patient and control groups.

When the correlations between ADMA and laboratory measurements were examined in the patient group, there was no correlation between ADMA and other parameters.

**FIGURE 3** The comparison of echocardiography parameters between the patient and control groups.

**FIGURE 4** The correlation between serum ADMA and tricuspid A.

**Hosted file**

`Table 1.docx` available at https://authorea.com/users/513565/articles/709952-the-effect-of-plasma-endocan-and-asymmetric-dimethyl-arginine-levels-on-endothelial-and-cardiac-functions-in-children-with-beta-thalassemia-major

**Hosted file**

`Table 2.docx` available at https://authorea.com/users/513565/articles/709952-the-effect-of-plasma-endocan-and-asymmetric-dimethyl-arginine-levels-on-endothelial-and-cardiac-functions-in-children-with-beta-thalassemia-major

**Hosted file**

Table 3.docx available at https://authorea.com/users/513565/articles/709952-the-effect-of-plasma-endocan-and-asymmetric-dimethyl-arginine-levels-on-endothelial-and-cardiac-functions-in-children-with-beta-thalassemia-major

**Hosted file**

Table 4.docx available at https://authorea.com/users/513565/articles/709952-the-effect-of-plasma-endocan-and-asymmetric-dimethyl-arginine-levels-on-endothelial-and-cardiac-functions-in-children-with-beta-thalassemia-major

**Hosted file**

Table 5.docx available at https://authorea.com/users/513565/articles/709952-the-effect-of-plasma-endocan-and-asymmetric-dimethyl-arginine-levels-on-endothelial-and-cardiac-functions-in-children-with-beta-thalassemia-major