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Original Article

Plasma Endocan as a Biomarker of Endothelial Dysfunction in Egyptian Children with Beta-Thalassemia Major: A Single-Center Analysis

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Abstract

Background: Beta-thalassemia major (β-TM) is a transfusion-dependent disorder associated with iron overload and endothelial dysfunction. Endocan is a proteoglycan secreted by endothelial cells and has been proposed as an indicator of endothelial activation and inflammation. Children with β-TM face various problems, such as glomerular and tubular degeneration, cardiomyopathy, and endothelial damage, which require regular monitoring using a reliable and practical method. This study aimed to evaluate plasma endocan levels in Egyptian children with β-TM.

Materials and Methods: A case-control study was conducted at the Medical Research Institute, Alexandria University. The experimental group consisted of 40 children with transfusion-dependent β-TM who were aged between 12 and 16 years, and the control group involved 40 age- and gender-matched healthy children. Clinical assessments and laboratory tests, including hemoglobin, serum ferritin, and C-reactive protein, were performed. Plasma endocan levels were measured using enzyme-linked immunosorbent assay (ELISA). Data were analyzed using SPSS version 20. Appropriate parametric or non-parametric tests, based on data distribution, were applied, and a p value of < 0.05 was considered statistically significant.

Results: Children with β-TM had significantly higher plasma endocan levels than controls (median 332 vs. 44 ng/L, $p < 0.001$). Endocan levels did not significantly differ based on gender ($p = 0.575$) or splenectomy status ($p = 0.986$). A significant difference was observed between hepatitis C virus (HCV)-negative and HCV-positive cases ($p = 0.005$), with higher endocan levels in HCV-positive patients. No significant correlation was found between endocan levels and age, ferritin, or transfusion frequency ($p > 0.05$).

Conclusion: Children with transfusion-dependent β-TM exhibited significantly higher plasma endocan levels, as compared to the healthy controls. Endocan can serve as a potential non-invasive biomarker of endothelial dysfunction in pediatric thalassemia.

Keywords: Anemia, Beta-Thalassemia, Endothelial cells, Proteoglycan

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Introduction

Thalassemias are a group of hereditary genetic disorders that are characterized by a decrease or absence of synthesis in one or more globin chains in the structure of hemoglobin (Hb) (1).

β thalassemia major (β-TM) is an autosomal recessive disorder resulting from the inheritance of null alleles (often β0 point mutations) in both HBB genes (chromosome 11p15.5) (2). It can lead to chronic hemolytic anemia, which can range in severity based on the level of deficiency of β-globin chain and the resulting aggregation of α-globin chains (3).

Children with β–TM require red blood cell transfusions and iron chelation treatment throughout their lives. Insufficient treatment can lead to tissue injuries and organ failures. Iron deposits, mainly in the heart, liver, and endocrine glands, can cause endothelial dysfunction (4). Iron toxicity is caused by free radicals produced by non-protein-bound, non-transferrin-bound iron, which accelerates the production of free hydroxyl radicals and facilitates tissue uptake (5).

Beta-thalassemia major in children is associated with early endothelial activation, mediated by oxidative stress and iron overload, which may be mitigated through timely therapeutic intervention. In adults, sustained endothelial injury due to impaired flow-mediated dilation can lead to chronic dysfunction and structural vascular changes with low probability for spontaneous recovery without prolonged treatment (6, 7).

Endocan, previously known as endothelial cell-specific molecule 1 (ECSM-1), is a proteoglycan secreted by the cells of vascular endothelium in a variety of organs. Pro-inflammatory cytokines and proangiogenic substances can promote endocan production. The levels of serum endocan are increased in case of endothelium inflammation, activation, and neovascularization. Studies have shown that diabetes mellitus, chronic renal disease, sepsis, hypertension, and acute coronary syndrome are among the conditions with endothelial dysfunction that have been linked to elevated serum endocan levels (8-12).

Children with β-TM are prone to diverse different health problems, such as glomerular & tubular degeneration, cardiomyopathy, and endothelial destruction, which need regular follow-up by a reliable and practical method (13). This study is to assess plasma endocan levels in Egyptian children with β-TM.

Material and Methods

A case-control study was conducted at the Medical Research Institute, Alexandria University. The research ethics committee approved the study (IRB number: E/C. S/N. R13/2023). The informed consents were signed by either of children's parents before their enrollment in this study.

Sample size: the sample size was calculated according to Khanmammadov et al. (2022) (14). The G*Power version 3.1.9.4 software was used, and a one-tailed Student's t-test with a statistical power of 0.80 was employed. Furthermore, an alpha of 0.05 and a medium effect size of 0.6 was considered. The calculated minimum sample size was 36 for each group.

Forty Egyptian children of both genders, aged 12 to 16 years old, were included and diagnosed with transfusion-dependent β-TM. Forty healthy children were considered as the control group; they were age- and gender-matched to the children in the experimental group. Serum C-reactive protein (CRP) levels were < 0.6 mg/dl (negative) for all children included in the study. Cases were recruited from attendees of the Pediatric Hematology Clinic, and the controls were recruited from the siblings of children attending the General Pediatrics Clinic at the Medical Research Institute of Alexandria University. Participants' enrollment was done from August 2023 to November 2024.

Patients were excluded from the study if they had other types of chronic hemolytic anemia, congenital heart diseases, cardiovascular diseases, hypertension, diabetes mellitus, or previously known renal or hepatic diseases. A detailed medical history was taken from each participant, participants' medical records were reviewed, and their physical examinations were performed.

Laboratory tests included complete blood counts and CRP. The circulating endocan levels of participants in each group were determined by using a commercially available enzyme-linked immunosorbent assay (ELISA). Hepatitis C virus

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(HCV) antibodies were assessed by Abbott Architect i1000SR immunoassay Analyzer, and serum ferritin levels were taken from patients' files (measured within one month before performing this study).

Endocan level was measured in the collected patient sera, using ELISA kits (BT LAB, Jiaxing, Zhejiang, China, Cat. No. E3160Hu), and all experiments were performed according to the manufacturer's instructions.

Statistical analysis

The Statistical Package for the Social Sciences (SPSS) software, version 20, Armonk, NY: IBM Corp, released 2011, was used for data analysis. Quantitative data were subjected to multiple tests to ensure thorough analysis. Mean and standard deviation tests were used to represent the normally distributed data, and were tested with student's t-tests. Furthermore, the median for non-normally distributed data was used, using the Mann-Whitney test for non-normally distributed data based on whether they were suitable for the type of data. Categorical variables were presented as frequency (percent), and the chi-square was used to explore the possible significant differences. The Spearman's correlation coefficient was employed to correlate two abnormally distributed quantitative variables. A p value of < 0.05 was considered significant.

Results

This case-control study, which was conducted at the Medical Research Institute, Alexandria University, included 40 cases with transfusion-dependent β-TM and 40 controls. Cases had a mean age of 14.1 ± 1.5 years with a male-to-female ratio of 1:1.5. The Hb level was significantly lower in the group with β-TM than the control group, while the number of white blood cells, neutrophils, and platelets, and the concentration level of ferritin of the group with β-TM were significantly higher than the control group ($p<0.001$). (Table I)

No statistically significant difference was observed in the number of lymphocytes and monocytes between the two groups. (Table I) As Figure I indicates, the mean level of endocan in the case group was significantly higher than that in the control group (332 vs. 44 ng/L, $p < 0.001$).

There was no difference in the endocan levels between males and females ($p=0.5$), splenectomized and non-splenectomized children ($p=0.9$), and children using different types of chelation therapy ($p=0.3$). Endocan level in HCV-positive cases was significantly higher than that in HCV-negative cases ($p=0.005$). Table II.

No significant correlation was observed between serum endocan levels and age or the rate of transfusion among cases. Additionally, as Table III shows, there was no significant correlation between the endocan level and ferritin level among cases.

Table I: A comparison between laboratory data of β-TM cases and the controls

Variable	Case (n = 40) mean ± SD (minimum - Maximum)	Control (n = 40) mean ± SD (minimum - Maximum)	*p* value
HB (g/dL)	8.4 ± 1.1 (6.7 – 10.8)	12.7 ± 0.6 (11.6 – 14)	<0.001
WBCs($\times10^9$/L)	11 ± 3.4 (4.5 – 17)	7.7 ± 1.6 (4.4 – 10.3)	<0.001
PLT($\times10^9$/L)	485.4 ± 169 (215 – 840)	273 ± 57 (166 – 410)	<0.001
Neutrophil %	53 ± 7.4 (32 – 70)	49.1 ± 4.3 (39 – 57)	0.007
Lymphocyte %	39.2 ± 7.2 (18 – 61)	37.1 ± 3.4 (31 – 44)	0.092
Monocyte %	5.8 ± 2 (2 – 10)	5.5 ± 1.8 (2 – 9)	0.522
Ferritin (ng/L)	3575.4 ± 1459.9 (1400 – 7123)	79 ± 30.8 (18 – 147)	<0.001

Hb: hemoglobin, n: number, PLT: platelets, WBCs: white blood cells, mean ± SD. Student t-test, the p value is significant at <0.05.

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Table II: The relationship between the endocan level and different parameters

Variable	Endocan (ng/L) Median (IQR) [Min. – Max.]	*p* value
Sex **Male (n=16)** **Female (n=24)**	 427.3 (259.2-978.7) [150.9 – 1243.6] 254.7 (168.4-651.6) [127.9 – 1734.8]	0.575
HCV status **Positive (n=3)** **Negative (n=37)**	 359.7 (174.9-348.8) [127.9 – 1734.8] 180.2 (188.3-949.8) [175.0 – 348.8]	0.005
Splenectomy **Yes (n=32)** **No (n=8)**	 337.3 (258.6-748.9) [127.9 – 1734.8) 326.5 (176.9-865.18) [157.1 – 1444.1]	0.986

HCV: Hepatitis C virus, n: number, IQR: Interquartile range, Min.–Max.: minimum-maximum Mann-Whitney test, the p value is significant at <0.05.

Table III: The relationship between the endocan level and various parameters

Endocan (ng/L) vs.	R	*p* value
Age (years)	-0.111	0.494
Rate of Transfusion	-0.106	0.516
HB (g/dL)	-0.046	0.780
WBCs($\times 10^9$/L)	-0.042	0.796
PLT($\times 10^9$/L)	-0.183	0.258
Neutrophil %	-0.023	0.887
Lymphocyte %	-0.077	0.636
Monocyte %	0.285	0.075
Ferritin (ng/L)	0.135	0.405

``Hb: hemoglobin, PLT: platelets, WBCs: white blood cells, the p value is significant at <0.05, (r) is Spearman's rank correlation coefficient.

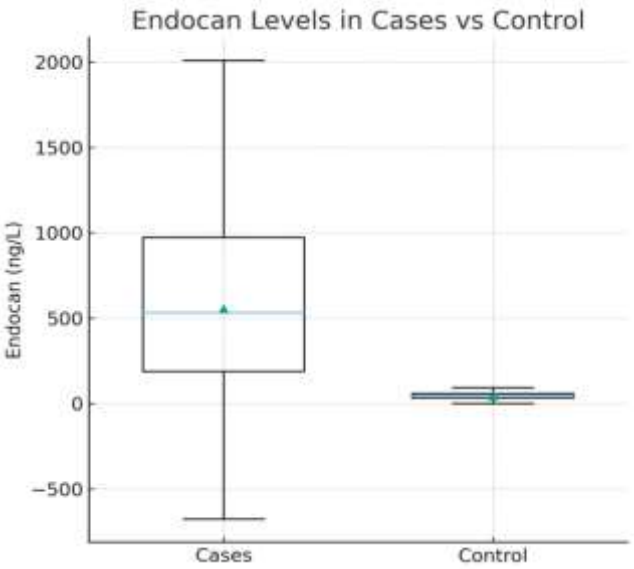

Figure 1. A comparison of the endocan level between Cases with β-TM and the Controls.

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Discussion

Endocan is one of the newly discovered proteoglycans produced by the vascular endothelial cells. It has been suggested that endocan is a sensitive molecule in activating the endothelium because of its low level in the endothelium under physiological conditions in comparison with its elevated level during inflammation, as well as angiogenesis (9).

Endocan induces the proliferation of the vascular smooth muscle cell. Therefore, it causes neointimal layer development at the stage of atherogenesis. In addition, it has been postulated that endocan might be a modulatory factor in leukocyte migration and adhesion to the endothelial layer (15, 16).

In the present study, statistically significant differences were observed in the level of serum endocan between patients with transfusion-dependent β-TM and the healthy control group.

In thalassemic patients, elevated serum endocan levels, attributable to endothelial dysfunction and inflammation, were associated with vascular complications including vasodilation, edema, coagulopathy, ischemic changes, and organ failure. It is well known that iron overload causes toxicity in many organs in patients with thalassemia.

Previous research on various diseases, including cancer, systemic inflammatory diseases, and cardiovascular diseases, has shown the links between the endocan level, endothelial dysfunction, and inflammation (11).

Khanmammadov et al. reported that there was no significant difference in the endocan level between cases with beta-thalassemia minor and the control group (14). Also, Botta et al. found that the endocan level can be high in transfusion-dependent beta-thalassemia patients (17).

The aforementioned studies, however, have been mostly done on adults, and the endocan level has been assessed in different age groups. In other words, to the present authors' knowledge, few relevant studies have been done on children with β-TM.

In this study, statistically significant differences were found in the levels of serum ferritin between children with thalassemia and children in the control group.

As the serum level increases in patients with thalassemia, hemosiderosis is an inevitable complication of prolonged transfusion therapy (18). This is because each unit of blood delivers 200 mg of iron to the tissue. Another cause is increased iron absorption from the gut (19), which results from transfusion therapy and increased absorption of dietary iron, and which is a severe complication of thalassemia major (20).

If left without treatment, the cumulative effect of iron overload causes significant morbidity and mortality. The excess free iron has the capability of catalyzing the synthesis of a large number of injurious molecules from regular metabolic end products, such as hydrogen peroxide and hydroxyl radical (21). The injurious reactive hydroxyl radical is free; therefore, it enters any cell and causes the destruction of deoxyribonucleic acid, proteins, and lipids (22).

Endocan has been postulated to be a marker of inflammatory endothelium dysfunction in endothelial-dependent diseases, such as cardiac diseases, conditions associated with sepsis, lung and renal diseases, and neoplasms (9).

In the present study, a statistically significant relation was found between the serum endocan level and positive HCV cases. In their case-control study, too, Tok et al. found that the serum endocan level in 19 patients with chronic hepatitis C (aged 55.0±12.8 years), as compared with the serum endocan level in controls, significantly decreased (23).

Hepatitis C infection can trigger an inflammatory response in the liver and possibly throughout the body. The higher levels of endocan observed in HCV-positive individuals are likely due to the virus triggering an inflammatory response that leads to activation of endothelial cells and promotion of endocan production. This can reflect endothelial cell dysfunction and systemic inflammation associated with chronic hepatitis (24).

Unlike the results of this study, Laloglu et al. reported a significant positive correlation between endocan levels and such inflammatory markers as

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ferritin in patients with coronavirus disease 2019 (COVID-19). In this study, no statistically significant correlation was observed between serum endocan and serum ferritin levels (25).

This can be explained by the different causes of elevated ferritin in both COVID-19 and thalassemia. Ferritin is a known inflammatory biomarker in COVID-19 (26), as serum ferritin is an acute phase reactant that increases with inflammation, and high ferritin levels can indicate an activated monocyte-macrophage system (27).

Aygunes et al. observed no correlation between endocan and serum ferritin in children with β-TM (28). Additionally, Cannavo et al. found no correlation between endocan and serum ferritin in children with multisystem inflammatory syndrome (29).

While Cetinkaya et al., however, reported a significant correlation between endocan and serum ferritin in patients with β-TM (13).

The present researchers faced a number of limitations in conducting this study. For instance, it was not possible to include a larger number of participants in this study. Furthermore, no follow-up was run to assess changes in endocan level over time, as it may be influenced by temporary conditions. Despite these, however, this study has some strengths, too. For instance, it is among the few studies that have been done to assess serum endocan in children with beta-thalassemia. Nonetheless, the present researchers advocate for larger, multicenter, and longitudinal studies to further explore the clinical utility of assessment of endocan level in guiding early therapeutic interventions and evaluating long-term vascular outcomes in pediatric thalassemia populations.

Conclusion

Children with transfusion-dependent β-TM exhibited significantly higher plasma endocan levels, as compared to the healthy controls. No significant correlations were found between endocan level and ferritin level, age, and transfusion rate, but a notable difference was observed between HCV-positive and HCV-negative patients. These findings can highlight the potential of endocan as a new inflammatory biomarker for endothelial activation and may be used to predict endothelial damage in pediatric thalassemia major that needs early intervention to prevent complications, especially cardiac disorders, in adulthood.

Availability of Data

Available upon reasonable request from the authors.

Ethical Considerations

The study was approved by the Medical Research Institute's Ethics Committee, Alexandria University (IRB number: E/C. S/N. R13/2023), and the confidentiality of patients' data was respected.

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During the preparation of this work the author(s) did not use AI.

Authors' Contributions

Nesrine A.Helaly: preformed design of the work, analysis and interpretation of data.

Mona Hassan: design of the work, writing, review, and editing of the manuscript.

Ahmed Afifi: design of the work, writing, review the manuscript.

Sally A.M.Saleh: design of the work, acquisition, analysis of data and writing.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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