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Assessment of serum endocan levels in patients with beta-thalassemia minor

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SUMMARY

OBJECTIVE: Beta-thalassemia minor is a blood disease caused by a hereditary decrease in beta-globin synthesis, frequently leading to hypochromic microcytic anemia. Formerly called endothelial cell-specific molecule 1, endocan is a proteoglycan released by vascular endothelial cells in many organs. Our aim was to investigate the relationship between the beta-thalassemia minor patients and the healthy control group in terms of serum endocan level.

METHODS: The study was performed in a total of 80 subjects. They were divided into two groups, the beta-thalassemia minor group (n=40) and the healthy control group (n=40). Serum endocan levels, age, sex, body mass index value, and tobacco use data of these groups were compared.

RESULTS: No statistically significant difference was detected between the two groups in terms of age, sex, and body mass index values (p>0.05). Endocan levels were measured to be 206.85±88.1 pg/mL in the beta-thalassemia minor group and 236.1±162.8 pg/mL in the control group with no significant difference between the groups in terms of serum endocan levels (p>0.05).

CONCLUSIONS: In our study, there was no change in endocan level in beta-thalassemia minor. This might be because serum endocan levels are affected by multi-factorial reasons. Serum endocan levels may be altered secondarily to decreased beta-globin chain, increased sympathetic activity due to anemia, or platelet dysfunction induced by oxidative stress in beta-thalassemia minor. Further multicenter studies involving more patients are necessary to demonstrate this.

KEYWORDS: Thalassemia. Anemia. Endothelial cells. Proteoglycan.

INTRODUCTION

Thalassemias are a group of hereditary genetic disorders characterized by the reduction or absence of synthesis of one or more globin chains in the hemoglobin structure. Beta-thalassemia minor (BTM) is characterized by hypochromic microcytic anemia caused by hereditary decrease in beta-globin synthesis[1]. Studies have reported that cerebrovascular and cardiovascular ischemic events might occur less in BTM patients. We believe that this might be due to decreased serum total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and triglyceride (TG) levels, mild anemia (due to low blood viscosity and microcytosis), low incidence of arterial hypertension, and hypofunctional platelets (PLTs) in BTM patients[2-6]. Formerly called endothelial cell-specific molecule 1, endocan is a proteoglycan released by vascular endothelial cells in many organs. Its synthesis is increased by proangiogenetic molecules and pro-inflammatory cytokines. Serum endocan levels are increased in endothelium activation (inflammation) and neovascularization. Studies have shown that serum endocan levels are increased in diseases with endothelial dysfunction such as chronic kidney disease, diabetes mellitus (DM), acute coronary syndrome (ACS), sepsis, and hypertension[7-11].

We aimed to show whether serum endocan levels affect the less frequent occurrence of cardiovascular and cerebrovascular ischemic events in BTM patients. Therefore, we investigated the serum levels of endocan, a marker of endothelial dysfunction between the BTM patients and the healthy control group, which was never performed in the literature earlier.

METHODS

Study group

This was a prospective, cross-sectional, case-controlled study that was initiated after a written approval was obtained from the Bezmialem Vakif University ethics committee and all subjects (approval no:71306642-050.01.04). A total of 80 subjects (40 patients with BTM and 40 in the control group) with similar age, gender, and body mass index (BMI) who applied to Bezmialem Vakif University, Faculty of Medicine, Internal

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Medicine Outpatient Clinic and met the inclusion criteria were included in the study.

Inclusion and exclusion criteria

Subjects aged between 18 and 65 years were included in the study. Patients with hypertension, DM, ischemic cardiac disease, cerebrovascular disease, chronic inflammatory autoimmune disease, malignancy, chronic pulmonary disease, thyroid dysfunction, and severe obesity (BMI >35 kg/m^2), and patients who are pregnant or lactating were excluded from the study.

Group classification

The mean corpuscular volume (MCV) values of the BTM subjects included in the study were <80 fL, and hemoglobin alpha 2 (HbA2) was ≥3.5%. Subjects who were not anemic (Hb≥13 g/dL for men and ≥12 g/dL for women) with normal MCV values were taken into the control group.

Blood assay

Venous blood samples collected into gel biochemistry tubes from all subjects between 8:00 a.m. and 9:00 a.m. after 12 h of fasting were centrifuged for 10 min at 2,100 rpm. Sera of all subjects were separated and aliquoted into Eppendorf tubes and kept at -80°C until the study day. On the study day, the serum samples were brought to room temperature, and routine biochemistry tests and endocan levels were studied in the medical biochemistry laboratory. Complete blood count (CBC) was studied on the same day when the blood was collected from the volunteers into tubes with K2EDTA.

The concentration of serum endocan level was measured by a specific commercial ELISA kit according to the manufacturer's instructions. Concentrations were determined using a spectrophotometric microtiter plate reader (Varioskan Flash Multimode Reader; Thermo, Waltham, MA, USA) at 450 nm optical density. Results were expressed as pg/mL.

Complete blood count was analyzed using Sysmex XT 1800i device (ROCHE2011, Kobe, Japan). Biochemical analyses were performed using COBAS 8000 device (ROCHE-2007, Tokyo, Japan) and COBAS-C system kits. Thyroid hormone levels were measured using Advia Centaur (Advia-2013-Tarrytown, USA) kits. Hemoglobin electrophoresis was performed by high-performance liquid chromatography method using the Shimadzu 20-A (Shimadzu-2013, Kyoto, Japan) device.

Furthermore, the homeostasis model assessment of insulin resistance index (HOMA-IR), a measure of insulin sensitivity, was calculated by multiplying the fasting insulin concentration (μU/mL) and fasting glucose concentration (mmol/L) and divided by 22.5[12].

Statistical analysis

Statistical Package for Social Sciences (SPSS), Windows 20.0 software, was used for the statistical analysis of the data. Quantitative variables were expressed as mean±standard deviation. The Mann–Whitney U test was used for the comparison of the quantitative variables between two groups. Student's t-test was used to compare the parametric variable between the patient and the control group, and the chi-square test was used for the comparison of the categorical variables. Bivariate correlation analyses were performed using Spearman's test. A p-value of <0.05 was considered to be significant.

RESULTS

A total of 80 subjects, 31 men (38.8%) and 49 women (61.2%), were included in the study. Mean age of the subjects was 36.99±12.29 years. There was no statistically significant difference between the mean age and mean BMI between the BTM and control group (p>0.05). Erythrocyte count [red blood cell count (RBC)], red distribution width (RDW), and HbA2 and hemoglobin F (HbF) values of the BTM group were statistically significantly higher than the control group (p=0.001). Hemoglobin (Hg) and hematocrit (Hct), HbA, and MCV values of the BTM group were found to be statistically significantly lower than the control group (p=0.001). No statistically significant difference was detected between the PLT and white blood cell (WBC) values of the groups (p>0.05; Table 1).

Endocan values were measured to be 206.85±88 pg/mL in the BTM group and 236.1±162.8 pg/mL in the control group with no statistically significant difference between the two groups (p>0.05; Table 1). Erythrocyte sedimentation rate (ESR) was statistically significantly lower in the BTM group compared with the control group (p<0.05; Table 1).

No statistically significant difference was detected between the groups in terms of serum glucose, creatinine, TC, TG, HDL-C, LDL-C, glycosylated hemoglobin (HbA1c) and HOMA-IR, transferrin saturation (TS), and thyroid-stimulating hormone (TSH) measurements (p>0.05; Table 1).

When the serum endocan levels, sex, and tobacco use data were compared between the two groups, no statistically significant difference was detected (p>0.05; Table 2).

No statistically and regression analysis-based significant relationship was detected between the BTM (+) and the control group in terms of age-endocan and, HbA-endocan relationships (p>0.05; Table 3).

Table 1. Evaluation of demographic characteristics and blood assay results in the beta-thalassemia minor and control group.

Groups			Test value
BTM (+) (n=40) Mean±SD		Control (n=40) Mean±SD	p
Age (year)	38.83±12.26	35.15±12.20	0.183
BMI (kg/m^2)	26.96±3.59	26.31±4.33	0.466
WBC (10^3/µL)	7.33±1.57	7.56±1.5	0.496
RBC (10^6/µL)	5.67±0.69	4.88±0.47	0.001*
Hb (g/dL)	11.28±1.38	13.91±1.58	0.001*
Hct (%)	36.22±4.12	41.66±4.32	0.001*
MCV (fL)	63.96±3.64	85.35±3.86	0.001*
PLT (10^3/µL)	238.9±61.47	251.28±49.88	0.326
RDW (%)	15.81±2.58	13.51±2.90	0.001*
HbA (%)	93.26±2.03	97.28±0.29	0.001*
HbA2 (%)	5.24±0.64	2.69±0.32	0.001*
HbF (%)	1.47±1.56	0±0.02	0.001*
Endocan (pg/mL)	206.85±88.1	236.1±162.8	0.321
CRP (mg/dL)	0.48±0.54	0.58±0.62	0.675
ESR (mm/h)	6.5±6.33	8.13±6.9	0.038*
Glucose (mg/dL)	94.03±26.42	88.93±9.92	0.421
Creatinine (mg/dL)	0.76±0.12	0.8±0.13	0.100
TC (mg/dL)	172.25±36.67	188.30±46.22	0.116
Triglyceride (mg/dL)	105.63±58.54	116.65±67.95	0.679
HDL-C (mg/dL)	51.58±12.93	54.53±27.98	0.497
LDL-C (mg/dL)	99.3±25.84	111±36.61	0.103
HbA1c (%)	5.41±0.54	5.34±0.31	0.462
AST (U/L)	19.85±10.55	19.13±6.24	0.977
ALT (U/L)	23.25±26.57	23.13±23.73	0.747
Transferrin saturation rate (%)	29.13±14.5	29.23±13.14	0.974
TSH (µIU/mL)	1.51±0.82	1.74±0.74	0.185
FT3 (pmol/L)	4.64±0.55	4.62±0.53	0.882
FT4 (pmol/L)	12.74±1.46	13±1.37	0.423
HOMA-IR	2.65±3.44	2.48±3.22	0.108

*Statistically significant. SD: standard deviation; BTM: beta-thalassemia minor; BMI: body mass index; WBC: white blood cell count; RBC: red blood cell count; Hb: hemoglobin; Hct: hematocrit; MCV: mean corpuscular volume; PLT: platelets; RDW: red blood cell distribution width; HbA: hemoglobin A; HbA2: hemoglobin alpha 2; HbF: hemoglobin F; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; TC: total cholesterol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; HbA1c: glycosylated hemoglobin; AST: aspartate amino transferase; ALT: alanine amino transferase; TSH: thyroid stimulating hormone; FT3: free triiodothyronine; FT4: free thyroxine, HOMA-IR: homeostasis model assessment insulin resistance.

Table 2. Evaluation of serum endocan levels by gender and smoking status between beta-thalassemia minor and control group.

			Endocan test value	
			Mean±SD	p
BTM (+)	Sex	Men (n=15)	188.8±80	0.369
		Women (n=25)	217.6±92.5	
Control	Sex	Men (n=16)	203.5±74	0.599
		Women (n=24)	257.8±200	
BTM (+)	Smoking	None (n=20)	205.0±93.9	0.899
		Present (n=20)	208.6±84	
Control	Smoking	None (n=20)	230.7±149	0.860
		Present (n=20)	241.5±178.8	

SD: standard deviation; BTM: beta-thalassemia minor.

Table 3. Relationship of endocan with age and laboratory results between the beta-thalassemia minor (+) and control group.'

	BTM (+)		Control	
	Endocan		Endocan	
	r	p	r	p
Age	0.182	0.260	0.141	0.385
HBA	-0.083	0.612	0.295	0.650
Glucose	0.077	0.639	0.101	0.535
Creatinine	-0.104	0.524	0.045	0.784
TC	0.101	0.534	0.67	0.681
Triglyceride	-0.061	0.710	0.109	0.504
LDL-cholesterol	0.034	0.835	0.087	0.592
HDL	0.185	0.253	-0.018	0.91
ESR	0.093	0.570	0.008	0.960
HbA1C	0.153	0.345	-0.209	0.196
HOMA-IR	-0.068	0.677	-0.095	0.558

SD: standard deviation; BTM: beta thalassemia minor; HbA: hemoglobin A; TC: total cholesterol; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; ESR: erythrocyte sedimentation rate; HbA1c: glycosylated hemoglobin; HOMA-IR: homeostasis model assessment insulin resistance.

DISCUSSION

In this study, we aimed to detect the serum endocan levels and the relationship of this marker with anthropometric, metabolic, and biochemical parameters between BTM patients and healthy control group. In our literature search, we did not find a study evaluating the relationship of endocan levels with BTM patients and anemia.

Studies have reported that cerebrovascular and cardiovascular ischemic events might occur less in BTM patients. It was

shown that this might be because of decreased TC, LDL-C, and TG levels, mild anemia (due to low blood viscosity and microcytosis), low incidence of arterial hypertension, and hypofunctional PLTs in BTM patients[2-6]. However, the exact pathophysiology is yet to be illuminated. Endocan is believed to be released from endothelium and plays an important role in vascular diseases, inflammation, and endothelium-dependent pathological disorders[7-11]. In this study, we aimed to show that endothelium damage might be less in patients with BTM, and, as a result, whether the serum endocan levels are low or not.

Some studies have detected low serum, TC, and LDL-C levels in BTM, and it was caused by excessive LDL-C uptake by the reticuloendothelial system due to accelerated erythropoiesis in BTM[4,13]. In their study, Agarwal et al. found that the incidence of myocardial infarction is lower in BTM, and this was thought to be caused by low blood viscosity due to mild anemia and microcytosis in BTM[3] . In their study, Cikrikcioglu et al. detected low levels of CD-40 ligand and soluble P-selectin and, PLT activation factors in BTM. They stated that hypofunctional PLT in BTM might have protective effects against cardiovascular and cerebrovascular ischemic diseases[6]. In a study, patients with the history of thromboembolic cerebrovascular event, cerebrovascular disease percentage, and arterial blood pressure were found to be less. Thus, the reason for cerebrovascular ischemic diseases occurring less in BTM was due to the effect of low arterial blood pressure[5].

In general, it is thought that cardiovascular and cerebrovascular ischemic events in patients with BTM might be low due to anemia, microcytosis, hyperviscosity, low TC and LDL-C levels, and incidence of low arterial pressure. In some other studies, serum endocan levels were higher in patients diagnosed with ACS and essential hypertension. It was stated that endocan might be a novel marker showing endothelial dysfunction, and the increase in the endocan level might be a risk for cardiovascular and ischemic events[10,14].

In their study on lipid profiles in patients with BTM and control subjects, Hashemieh et al. detected significantly lower levels of TC and LDL-C in BTM. In the same study, when the subjects were classified by age, no significant difference was detected between the BTM patients and the control group for the subjects aged <25 years in terms of cholesterol levels [i.e., TC, TG, LDL-C, HDL-C, and very low-density lipoprotein cholesterol (VLDL-C)]. In the BTM group aged from 26 to 40 years, TC and LDL-C levels were significantly lower, and in the BTM group aged >40 years, only LDL-C level was significantly lower[4]. In their study, Selek et al. detected significantly lower LDL-C levels in BTM patients, and did not detect a significant difference for TC, TG, and HDL-C levels[15]. Thus, it can be shown that regional ethnicity, diet types, age, and genetic differences may affect the cholesterol levels in BTM. In our study, there was no statistically significant difference between the groups in terms of TC, TG, LDL-C, and HDL-C levels, because our study is a single-centered study with less number of patients.

Normally, inflammatory cytokines [i.e., interleukin 1 (IL-1) and, tumor necrosis factor alpha (TNF-α)] secreted after sepsis and inflammation induce endocan expression. The blood levels of this proteoglycan might reflect treatment response, and the presence and the severity of inflammation. Therefore, endocan has a role in endothelial dysfunction during the inflammatory process[9,16]. We also believe that endocan levels might be low in BTM as the inflammatory process is not involved in the etiopathogenesis of BTM.

Anemia might increase the activity of sympathetic nervous system[17,18]. Selek et al. detected that oxidative stress is increased due to anemia in BTM[15]. Oxidative stress increase in BTM might impair the structure of membrane glycoproteins in PLT and might cause a decrease in the PLT functions. PLT dysfunction might be congenital or acquired in BTM. Oxidative stress may also cause acquired PLT dysfunction[6].

Botta et al. found the endocan level to be high in transfusiondependent beta-thalassemia patients (beta-thalassemia major)[19]. This may be due to direct or indirect endothelial damage caused by chronic hemolysis and chronic iron overload in patients with β-thalassemia major. In our study, however, there was no significant change in endocan level in BTM patients. According to the study of Botta et al., it can be considered that our study was designed in a different thalassemia group, and there was no chronic hemolysis and iron overload. This indicates that endocan release might be affected in BTM due to congenital globin chain impairment, sympathetic activity, PLT dysfunctions, oxidative stress, or other factors.

Chronic inflammation also plays a role in the pathogenesis of atherosclerosis. In clinical practice, ESR is an acute phase marker used to show inflammation. In their cohort study in 1,679 patients, Natali et al. have shown that high ESR level is an independent marker of coronary atherosclerosis associated with cardiac mortality[20]. ESR might increase in macrocytosis, and chronic disease anemias developed secondarily to many illnesses (e.g., infection, inflammation, and malignancy) usually due to underlying diseases. ESR is decreased in polycythemia and some erythrocyte disorders (i.e., sickle cell anemia, microcytosis spherocytosis, and acanthosis)[21,22]. In our study, ESR was found to be significantly lower in the BTM group compared with the control group ($p<0.05$). We believe that, this is caused by the increase of total erythrocyte count in BTM.

The most important limitations of our study were as follows: (1) less number of subjects in the BTM and the control group were involved and this is a single-centered study and (2)

we could have used more objective parameters to demonstrate endothelial dysfunction (flow-mediated vasodilation).

CONCLUSION

In our study, no significant difference was detected between the BTM patients and the control subjects in terms of serum endocan levels. This might be because serum endocan levels are affected from multi-factorial reasons. Serum endocan levels may be altered secondarily to decreased beta-globin chain, increased sympathetic activity due to anemia, or PLT dysfunction induced by oxidative stress in BTM. Further multicenter studies involving more patients are necessary to demonstrate this.

AUTHORS' CONTRIBUITIONS

NK: Data curation, Writing – review & editing. **MZ:** Conceptualization, Investigation, Project administration, Writing – original draft. **OFO:** Formal Analysis, Methodology. **CK:** Resources, Validation. **MK:** Supervision, Data curation, Software. **MÇ:** Validation, Visualization, Funding acquisition.

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