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SELÇUK TIP DERGİSİ

Beta-Talasemi Majorlu Çocuk Hastalarda Karotis İntima Media Kalınlığı ve Paroksanaz Aktivitesi

Carotid Intima-Media Thickness and Paraoxonase Activity in Beta-Thalassaemia Major Children.

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Özet

Bu çalışmanın amacı Beta talesemi majorlu (BTM) çocuk hastalarda paroksanaz (PON 1) ve karotis intima media kalınlığının(KİMK) arasındaki ilişkinin değerlendirilmesidir. 50 BTM hastası (7.2±5.3 yıl, 34 erkek ve 16 kız) ve 35 kontrol (7.9±2.1 yıl, 23 erkek ve 12 kız) çalışmaya alındı. Tüm olgularda serum PON1 ve KİMK ölçüldü. Ortalama KİMK BTM li hastalarda kontrol grubuna göre anlamlı artmıştı (P<0.001). Tersine serum PON1 aktivitesi düşük olarak bulundu (<0.05). PON1 KİMK ile negatif karelasyondaydı (r=-0.642, p<0.0001). Yaş KİMK ile positif koreleydi (r=0.741, p<0.0001). BTM li hastalarda PON1 aktivitesi ve KİMK arasında anlamlı korelasyon bulundu. Erişkin hastalığı olarak bilinen aterosklerozun çocukluk çağında başlayabileceği gösterilmiştir.

Anahtar kelimeler: Beta talesemi major, paroksanaz, KİMK

Abstract

The aim of this study was to research the relationship between the difference in carotid intima media thickness and paraoxonase (PON1) in beta-thalassaemia major (BTM) children. We recruited fifty BTM patients (7.2±5.3 years, 34 boys and 16 girls) and 35 controls (7.9± 2.1 years, 23 boys and 12 girls) consecutively. In all subjects, serum PON1 activity and CIMT were measured. Mean CIMT was significantly increased in BTM patients relative to controls (P<0.001). Conversely, serum PON1 activity was decreased (<0.05). PON1 activity was negatively correlated with CIMT (r=-0.642, p<0.0001). Age was positively correlated with CIMT (r=0.741, p<0.0001). A significant correlation was determined between PON1 activity and CIMT in patients with BTM. This indicates that atherosclerosis, which is known as an adult disease, may start in childhood.

Key words: beta-thalassaemia major-paraoxonase-CIMT

INTRODUCTION

Patients with beta-thalassaemia major (BTM), which is a disease of red blood cells, may present with clinical complications in several organ systems, which results from the oxidative stress induced by iron overload (1). With the increased life span of BTM patients, coronary artery disease may emerge as one of the important cardiovascular complications (2). Studies have suggested a link between iron load and risk of atherosclerosis. Endothelial dysfunction, which is an important precursor of atherosclerosis, was found in BTM patients due to peroxidative tissue injury because of continuous blood transfusions (3). High resolution ultrasound is a reliable, non invasive method for detecting early structural and functional atherosclerotic changes in the arterial wall (4). Increased carotid artery intima media thickness (CIMT) is a structural marker for early atherosclerosis and it correlates with the vascular risk factors and to the severity and extent of coronary artery disease (5). In recent years, increasing evidence suggests that the oxidative modification of LDL is the key step in the sequence of events leading to atherogenesis-related vascular alterations (6). It has been demonstrated that paraoxonase deficiency, is related to increased susceptibility to LDL oxidation and development of atherosclerosis (7). The aim of this study was to evaluate the carotid IMT and PON-1 activities in patients with BTM compared with normal controls.

MATERIALS AND METHODS

Subjects

A total of 50 (34 boys and 16 girls) patients with BTM (7.2±5.3 years) and 35 (23 boys and 12 girls) healthy controls (7.9± 2.1 years) were included in this study. Patients were regularly interviewed and examined by a staff of physicians at 15 days to 1 month intervals. Serum ferritin was measured every 4 months, and cardiac, endocrinologic, and hepatologic evaluations were performed once a year. The patients received approximately 15mL of packed red blood cells per kilogram of their body weight at each transfusion to maintain hemoglobin levels above 9.5 g/dL. This value is that we tried to keep above but as the patients' sociocultural and socioeconomic levels are low in our region, during follow-up visits various unwanted problems arose from the patients themselves. Therefore, some patients did not attend for check-ups or came late so this was the reason for the low level of our patients' mean hemoglobin levels. Deferoxamine was applied as chelation therapy. The therapy did not involve intake of ascorbate. Their diagnoses were confirmed through hemoglobin electrophoresis, blood count results, and physical examination. As most thalassemic patients require intermittent blood transfusions, blood was collected as late as possible, at least 3 to 4 weeks after the last transfusion. Hemoglobin electrophoresis was performed on all patients and healthy controls to exclude the b-thalassemia trait.

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They all had normal blood counts and hemoglobin electrophoresis results. Hemoglobin electrophoresis was performed using alkaline cellulose acetate electrophoresis densitometry. Complete blood count was performed using by Celdyne 3700 Haematology Analyser (Abbott). Serum ferritin was measured using an automated chemiluminescence auto-analyzer (Roche). The study protocol was carried out in accordance with the Helsinki Declaration as revised in 1989. Written consent was obtained from all parents before their children's participation in the study. The study was approved by the local ethics committee.

Exclusion Criteria; Exclusion criteria included usage of supplemental vitamins, smoking, presence of diabetes mellitus, coronary artery disease, rheumatoid arthritis, malignancy, hypertension, hyperlipidemia, systemic or local infection, acute chronic liver diseases, renal dysfunction, and iron deficiency anemia.

Blood sample collection

Blood from BTM patients was collected just before the transfusion. Control blood was obtained from healthy individuals, who were not taking any medication. Blood samples were obtained after an overnight fasting state, and collected into empty tubes and stored immediately on ice at 40C. The serum samples were then separated from the cells by centrifugation at 3000 rpm for 10 minutes, and lipid parameters and the enzymes activities were measured freshly. Remaining serum portions were stored at -800C for a time no longer than 6 months (8) and used to analyze measurement of paraoxonase and arylesterase activities.

Measurement of paraoxonase activities

Paraoxonase activities was measured by using paraoxon and phenylacetate substrates. The rate of paraoxon hydrolysis (diethyl-p-nitrophenylphosphate) was measured by monitoring the increase of absorbance at 412nm at 370C. The amount of generated p-nitrophenol was calculated from the molar absorptivity coefficient at pH 8, which was 17,000/M/cm (9). Paraoxonase activity was expressed as U/L serum. Phenylacetate was used as a substrate to measure the arylesterase activity. Enzymatic activity was calculated from the molar absorptivity coefficient of the produced phenol, 1310/M/cm. One unit of arylesterase activity was defined as 1 μmol phenol generated per minute under the conditions mentioned above and expressed as U/L serum (10). Paraoxonase phenotype distribution was determined by a double substrate method that measures the ratio of paraoxonase activity (with 1M NaCl in the assay) to arylesterase activity, using phenylacetate (9).

Sonographic study

All of the sonographic examinations were performed by the same physician , who was unaware of the subject's clinical status throughout the study. Each subject was studied in the morning hours (8:00 a.m. to 10:00 a.m.) after having abstained from alcohol, caffeine, tobacco and food for 8 hours before the examination. None of the participants was using vasoactive drugs. Studies were performed in a quiet, temperaturecontrolled room (20–250C). Images were obtained by high resolution Doppler ultrasonography (Logiq 7 Pro; General Electric, Milwaukee, WI) with a 12-MHz linear-array transducer. Sonographic examinations evaluated manually by the same investigator to avoid interobserver variations. Bilateral assessment of wall thickness was made in the CCA. IMT was measured as the distance from the leading edge of the first echogenic line to that of the second echogenic line. The first line represents the lumen–intima interface, and the second line the collagen-containing upper layer of tunica adventitia. IMT measurement of both the right and left CCA was performed at three points on the far walls in each CCA from 2 cm proximal to the bifurcation of the CCA. The three locations were then averaged to produce the mean IMT for each side. The average of the two sides was considered the patient's overall mean CIMT.

All data analysis was conducted using SPSS version 11.5 (SPSS Inc., Chicago, Illinois, USA), with group parameters expressed as means ± standard deviations. Between-group comparisons were conducted by independent samples Student's t-tests for continuous variables. Bivariate correlations were performed using Pearson's correlation analysis, for which an a priori designation system was adopted of: $R < 0.40$, weakly correlated; $R = 0.40–0.69$, moderately correlated; and $R > 0.70$, strongly correlated.

RESULTS

Demographic, clinical data and lipid profile of BTM patients and controls are summarized in Table 1. There were no significant differences between BTM patients and controls with respect to age and sex (all $P>0.05$) (Table 1). There were significant differences between BTM patients and controls with respect to height, weight, and body mass index (all $P<0.05$) (Table 1). There were significant differences between BTM patients and controls with respect to lipid profile, serum iron, hemoglobin, hematocrit, mean corpuscular volume, and ferritin (Table 1). Paraoxonase activities were significantly lower in patients with BTM than controls (Table 1). The CIMT of thalassemic patients was significantly increased

Table 1. Comparision of laboratory findings, demographical and clinical characteristics in BTM patients and controls.

	BTM (n:50)	Controls (n:35)	p value
Age (years)	8.8±3.9	9.0±4.06	>0.05
Sex (boy/girl)	32/18	22/13	>0.05
Height (cm)	119.3±19.2	130.0±21.2	<0.05
Weight (kg)	24.7±8.2	31.8±9.8	<0.001
BMI (kg/m2)	16.9±2.1	18.6±1.8	<0.001
Hb (g/dL)	8.4±1.4	12.1±1.4	<0.0001
Hct (%)	24.2±4.4	35.8±4.4	<0.0001
MCV (fL)	79.3±4.4	84.4±4.6	<0.0001
Serum iron (mg/dL)	164.1±63.2	79.7±18.6	<0.0001
Ferritin (mg/L)	3548.4±2025.7	47.5±20.0	<0.0001
Total cholesterol (mg/dl)	109.3±25.6	175.5±39.1	<0.0001
Triglyceride (mg/dl)	156.4±65.6	137.2±114.3	<0.05
LDL cholesterol (mg/dl)	53.4±22.7	91.8±27.6	<0.0001
HDL cholesterol (mg/dl)	25.5±5.8	54.0±15.2	<0.0001
Paraoxonase (U/L)	85.7±56.8	127.3±68.3	<0.05

Table 2. Common carotid artery IMT in BTM patients and controls.

	BTM patients (n:50)	Controls (n:35)	p value
Right CCA IMT (mm)	0.47±0.04	0.40±0.03	p<0.001
Left CCA IMT (mm)	0.47±0.05	0.40±0.04	p<0.001
Averaged CCA IMT (mm)	0.47±0.05	0.40±0.03	p<0.001

compared to controls (Table 2). The mean CIMT was (0.47±0.05 and 0.40±0.03 mm) in BTM patients and controls, respectively (p<0.001) PON1(r=-0.642, p<0.0001) activity were negatively correlated with CIMT and age (r=0.741, p<0.0001) was positively corelated with CIMT.

DISCUSSION

Atherosclerosis is a chronic process that affects all layers of arterial wall. The thickening of arterial wall is prominent particulary in intima media. A significant associated has been determined between IMT thickness and atheosclerosis(11). CIMT is usually measured from B-mode ultrasound images on which two echogenic lines representing the lumen–intima interface and the media–adventitia interface can be identified (12). As such, CIMT is a composite of intima and medial wall thickness. Measurement of IMT of the CCA by ultrasound is a highly reproducible method for the evaluation of early atherosclerosis (13). Cheung et al. studied 20 BTM patients and found an increase in their CIMT compared to controls (14). Our study provides evidence of premature atherosclerosis indicated by increased CIMT in young BTM patients compared to controls. Cell-free Hb has been implicated in mediating vascular dysfunction in thalassemic patients by limiting nitric oxide bioavailability, which acts in combination with endothelial dysfunction to increase the risk of premature atherosclerosis (14,15). Oxidative modification of LDL which is mainly related to iron overload, is the key step in the sequence of events leading to atherogenesis-related vascular alterations and increased CIMT (3). Modified LDL is internalized in monocyte-derived macrophages through cell surface scavenger receptors, an event that leads to foam cell formation. Infiltration and deposition of these cells in the arterial wall are the initiating steps in the development of atherometous plaque (16). Increased oxidative stress and decrease in antioxidants may have roles in both the pathogenesis of the disease and the development of atherosclerosis in BTM. PON activity was decreased in BTM (17). Activities of PON1 enzymes was also lower in the BTM group in the present study. LDL oxidation in arterial walls is believed to have an important role in atherogenesis (18). PON and ARE are esterase enzymes that have antioxidant characteristics. It is well known from epidemiological data that HDL exerts cardioprotective properties through its anti-oxidant activity, which is largely maintained by PON (19). PON hydrolyzes organophosphates and LPO products, and neutralizes the harmful effect of lipid peroxides in LDL. Thus, PON1 prevents the acceleration of atherosclerosis (20). Serum PON activity is generally considered to vary in response to the consumption of PON1 for prevention of oxidation (21). We have no data on oxidative stress in this study, however, the decrease in serum PON activity in BTM patients might be resulted from the increased inactivation of PON1 according to the increased generation of reactive oxygen species in BTM (3).

Kumon et al. reported that serum paraoxonase activity was downregulated by interleukin-1 and tumor necrosis factor-a. As we previously observed that proinflammatory cytokines of interleukin-1 (IL-1) and tumor necrosis factor-a (TNF-a) downregulated PON1 mRNA expression in HepG2 cells (22). Hahalis et al. reported that interleukin-6 and tumor necrosis factor-a were decreased in BTM. The reduction of PON1 production by liver with cytokines might be responsible for the decreased serum PON activity in BTM patients (3). A significant correlation was found serum paraoxonase activity and CIMT. In our opinion, the evaluation of the serum PON activity along with CIMT may be important in patients with BTM in consideration of the evaluation of coronary artery disease risks. This is the first study underlining this issue and reports decreased levels of PON1 activity and increased CIMT. On the other hand, in this study a significant positive correlation was found between age and CIMT. This correlation possibly was due to increased total tranfusion number during advancing age. In patients with low BTM, total cholesterol, HDL-cholesterol, LDL-cholesterol levels , the triglyceride levels were high (3,17). However, another study reported that the levels were the same in both study group and the control group (23). In the study group, total cholesterol, HDL-cholesterol, LDL-cholesterol levels were lower and the triglyceride level was higher than that of the control group.

LDL-C lowering effect of thalassemia is a well-known entity in all forms of thalassemia syndromes (24). Increased uptake of LDL by the bone marrow to provide cholesterol for the increased proliferation of erythroid progenitor cells and increased production of inflammatory cytokines that reduce the hepatic secretion and increase the catabolism of LDL has been suggested to be responsible from the low LDL-C levels observed in thalassemia patients (24). HDL may affect the active site of the PON1 enzyme protein, resulting in a decrease in serum PON activity in BTM. HDL was rapidly cleared from the circulation during inflammation (25). In conclusion, A significant correlation was determined between a decrease in paraoxonase activities and an increase CIMT in patients with BTM. This indicates that atherosclerosis, which is known as an adult disease, may start in childhood. These findings show that it might be beneficial for children who are being followed-up from a diagnosis of BTM to also be evaluated in respect of the development of atherosclerosis.

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