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Coronary Microvascular function, Peripheral Endothelial Function and Carotid IMT in beta-thalassemia minor

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ABSTRACT

Higher prevalence of cardiovascular disease in Thalassemia patients have been known. Potential mechanisms are enhanced platelet activation, LDL oxidation, macrophage activation, and increased nitric oxide destruction. We have investigated coronary flow reserve (CFR), brachial artery flow mediated dilation (FMD) and Carotid intima-media thickness (IMT) in patients with Beta thalessemia minor (BTM).

Methods: Forty patients with BTM and 35 healthy control subjects were included. In all subjects CFR, brachial artery FMD, carotid artery IMT were measured.

Results: CFR measurements: Coronary baseline diastolic peak flow velocity (DPFV) of left anterior descending coronary artery (LAD) was significantly higher in the BTM group (23.8 ± 3.9 vs. 22.1 ± 3.0, P = 0.04). However, hyperemic DPFV was significantly lower (61.1 ± 13.0 vs. 68.2 ± 14.2, P = 0.02), and CFR was significantly lower (2.57 ± 0.46 vs. 3.07 ± 0.48, P < 0.0001) in the BTM group than that in the control group.

Brachial artery FMD and carotid IMT measurements: Percent FMD measurements were significantly lower in the BTM group than that in the controls (6.22 ± 4.29 vs. 8.10 ± 4.00, P = 0.01). Carotid IMT measurements were significantly but slightly higher in the BTM group than that in the controls (0.57 ± 0.07 vs. 0.54 ± 0.04, P = 0.04).

Conclusion: CFR reflecting coronary microvascular function and brachial artery FMD are decreased, and carotid IMT is increased in patients with BTM.

Introduction

Beta-thalassemia is a group of inherited hemoglobin disorders ranging in severity from clinically silent heterozygous beta-thalssemia to severe transfusion-dependent thalassemia major. There have been a controversy over the relationship between coronary artery disease (CAD) and the thalassemia. Decrease in hemoglobin levels in anemia has been reported as an independent risk factor for cardiovascular disease. Severe iron overload in patients with beta-thalassemia major may actually be a risk factor for atherosclerosis [1–4]. An increase in PF3 activity in thassaemic patients due to abnormal erythrocytes leads to activation of the coagulation mechanisms. Thus, procoagulant milieu in thalassemia may participate in accelerated atherogenesis [5]. Potential mechanisms are believed to include enhanced platelet activation, LDL oxidation, macrophage activity stimulation, and increased nitric oxide destruction in the context of oxidative stress and hemolysis [6–9]. Some studies also suggest that impaired glucose tolerance and hypertrigliseridemia is more prevalent in beta thalassaemia [10]. On the contrary, lower incidence of acute myocardial infarction has recently been reported in thalassemia traits [11,12]. However, this protective effect was observed only in males [13,14].

Beta-thalassemia minor (BTM) is a clinical definition applied to thassemia patients who present a milder clinical course than those with beta-thalassemia major, with a less marked anemia that does not require treatment with regular blood transfusions [15]. A reduction in cardiovascular risk factors, particularly serum cholesterol levels [16], lower blood viscosity due to decreased haematocrit and hemoglobin levels [17], and a lower incidence of arterial hypertension [14] has been proposed to be responsible for the protective role of BTM from cardiovascular diseases [18]. There are not any study on vascular function, systolic/diastolic heart function and coronary microvascular function in these patients. This study provides preliminary data on vardiovascular function of patients with BTM.

Impaired flow mediated dilatation (FMD) of the brachial artery is thought to detect early stages of atherosclerosis because of its association with atherosclerotic risk factors and prognosis [10]. Likewise, intima-media thickness (IMT) of the carotid artery wall is linked with cardiovascular risk factors, including fibrinogen levels and blood viscosity as well as iron related oxidative stress [10,19–21]. Carotid IMT is related both with incident and prevalent cardiovascular disease and is accepted measure of subclinical atherosclerosis [19]. Coronary flow

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reserve (CFR) measurement is used both to assess epicardial coronary arteries and to examine the integrity of coronary microvascular circulation. Impairment of endothelial function and reduced CFR, which reflects coronary microvascular function, has been shown to be early manifestation of atherosclerosis and coronary artery disease [22,23]. In subjects with normal coronary arteries or mildly diseased coronary arteries, prognostic importance of CFR with respect to atherosclerosis has been emphasized [24]. Recently, in several studies, feasibility of second harmonic transthoracic Doppler echocardiography (TTDE) for evaluating CFR has been validated in evaluating CFR in the middle to distal portion of the LAD [22,25].

Therefore, in the present study we aimed to investigate CFR reflecting coronary microvascular function, peripheral endothelial function via FMD, and Carotid IMT in patients with BTM.

Methods

Study Population

Forty patients with BTM and Thirty-five healthy volunteers matched for age, sex and body-mass index (BMI) were included in this study. Patients who fulfilled the inclusion and the exclusion criteria were selected from the hematology out-patient clinic. Diagnosis of BTM was made in the case of hypochromic and microcytic hemoglobins, normal serum ferritin levels, and elevated serum levels of HbA2 (>4.0%) [26]. Hb electrophoresis was performed using Helena gel electrophoresis Beckmann densitometry. Complete blood count was performed using by Celdyne 3700 Haematology Analyser (Abbott®). Serum ferritin was measured using an automated chemiluminescence autoanalyzer (Roche®).

In each subject, a complete physical examination was performed, with particular attention to peripheral arterial pulses and carotid bruits. Each subject was questioned about major CV risk factors including family history of coronary artery disease (CAD), current smoking status, alcohol consumption, and diabetes mellitus. Family history of CAD was obtained by questioning the subjects about CAD in first-degree male relatives before 55 years and in female relatives before 65 years of age. Age, gender, and BMI were recorded. Fasting blood glucose, total cholesterol, HDL-cholesterol, LDL-cholesterol, and triglyceride levels were recorded. Plasma levels of C-reactive protein were measured by use of a highly sensitive sandwich Elisa technique.

Inclusion and Exclusion Criteria

Inclusion criteria included 18–55 years of age and having BTM. Exclusion criteria included presence of a valvular or congenital heart disease; cardiac rhythm other than sinus; previous myocardial infarction; hypo or hyperthyroidism; chronic obstructive pulmonary disease or cor pulmonale; systemic diseases such as collagenosis, hemolytic, hepatic, and renal diseases or any disease that could impair CFR (e.g., hypertrophic cardiomyopathy and diabetes mellitus: fasting plasma glucose level measured on three separate days in a week >126 mg/dL [7.0 mmol/L] or impaired oral glucose tolerance test: fasting plasma glucose <126 mg/dL [7.0 mmol/L] but 2-h plasma glucose after a 75-g oral glucose challenge >140 mg/dL [7.8 mmol/L]), family history of CAD, excessive alcohol consumption (>120 g/day), taking any vasoactive drug, and current smoking. Subjects were excluded from the study if they had ST segment or T wave changes specific for myocardial ischemia, Q waves, and incidental left bundle branch block on ECG. Individuals were also excluded if they had triglyceride levels >4.56 mmol/L (400 mg/dL), body mass index (BMI) greater than 35 kg/m^2, or left ventricular mass index (LVMI) ≥125 g/m^2 for men and 110 g/m^2 for women.

Written informed consent was obtained from each subject. The institutional ethics committee approved the study protocol. The study was conducted according to the declaration of biomedical researches on human subjects.

Echocardiographic Examination

Each subject was examined using an Acuson Sequoia C256® Echocardiography System equipped with a 3V2c broadband transducer with second harmonic capability (Acuson, Mountain View, CA, USA). Two-dimensional, M-mode, and subsequent standard and pulsed tissue Doppler echocardiographic examinations were performed on each subject in the lateral decubitus position. The echocardiographic images were recorded on VHS videotapes. Diastolic and systolic interventricular septal (IVS) thickness, posterior wall (PW) thickness, and left ventricular end-diastolic (LVDD) and left ventricular end-systolic (LVSD) diameters were measured on the parasternal long-axis views. All measurements were performed on M-mode images.

The pulsed Doppler sample volume was positioned at the mitral leaflet tips. Early diastolic peak flow velocity (E), late diastolic peak flow velocity (A) and E/A ratio, and E-wave deceleration time (DT) were measured by transmitral Doppler imaging.

The Doppler tissue-imaging (DTI) program was set to the pulsed-wave Doppler mode. Filters were set to exclude high-frequency signals, and the Nyquist limit was adjusted to a velocity range of −15 to 20 cm/s. Gains were minimized to allow for a clear tissue signal with minimal background noise. All DTI recordings were obtained during normal respiration. A 5-mm sample volume was placed at the apical four-chamber view on the lateral corner of the mitral annulus [27]. The resulting velocities were recorded for 5–10 cardiac cycles at a sweep speed of 100 mm/s, and stored on VHS videotape for later analysis. The following measurements were determined as indexes of regional systolic function: peak velocities (cm/s), time velocity integral of myocardial systolic (*S*m) wave. Myocardial early (*E*m) and atrial (*A*m) peak velocities (cm/s) and *E*m/*A*m ratio, and *S*m-*E*m duration (isovolumic relaxation time: IVRT) were measured, as the time interval occurring between the end of *S*m and the onset of *E*m, were determined as diastolic measurements. All diastolic parameters were measured in three consecutive cardiac cycles and averaged. The same investigator blinded for clinical data performed the echocardiography, and two cardiologists blinded for subjects' data analyzed the echocardiogram recordings.

CFR Measurement

Each subject was examined using an Acuson Sequoia C256® Echocardiography System equipped with a 3V2c and a 5V2c broadband transducers. (Acuson Corp, Mountain View, Calif, USA). All subjects were examined after a 12-hour fast and after they had abstained from caffeine- or xanthine-derivative-containing drinks for at least 12 h before the measurements. Visualization of the distal left anterior descending (LAD) coronary artery was performed using a modified, foreshortened, 2-chamber view obtained by sliding the transducer on the upper part and medially from an apical 2-chamber view to reach the best alignment to the interventricular sulcus. Subsequently, coronary flow in the distal LAD was examined by color Doppler flow mapping over the epicardial part of the anterior wall, with the color Doppler velocity range of 8.9–24.0 cm/s. The color gain was adjusted to provide optimal images. The acoustic window was placed at approximately the midclavicular line, in the fourth and fifth intercostal spaces, with the subject in the left lateral decubitus position [28]. The left ventricle was imaged on the long-axis cross-section, and the ultrasound beam was then inclined laterally. Next, coronary blood flow in the LAD (middle to distal) was searched by color Doppler flow mapping. All subjects had Doppler recordings of the LAD with a dipyridamole infusion at a rate of 0.56 mg/kg over 4 minutes. All subjects had continuous heart rate and electrocardiographic monitoring as well as blood pressure recordings at baseline, during dipyridamole infusion, and at recovery. Echocardiographic images were recorded on VHS videotapes. Two experienced echocardiographers, who had been blinded to the clinical data, analyzed the recordings. By placing the sample volume on the color signal, spectral Doppler of the LAD showed the characteristic biphasic flow pattern with larger diastolic

and smaller systolic components. Coronary diastolic peak velocities were measured at baseline and after dipyridamole by averaging the highest 3 Doppler signals for each measurement. CFR was defined as the ratio of hyperemic to baseline diastolic peak velocities [28].

IMT and FMD Measurement

A high-resolution 7.5-MHz linear array ultrasound transducer (attached to a Hitachi EUB 6500, Japan-2003) was used to measure brachial FMD and carotid IMT. All subjects were examined after a 12-hour fast and after they had abstained from caffeine- or xanthine-derivative-containing drinks for at least 12 h before the measurements. At least 2 days after the CFR measurement the study subjects were admitted for IMT and FMD measurements. After baseline measurements of the brachial artery had been recorded, the cuff was placed proximal to the section of brachial artery, inflated to 250 mm Hg (or 50 mm Hg higher than systolic blood pressure), and kept at the same pressure for 4.5 min to create forearm ischemia. Subsequently, the cuff was deflated, and the arterial diameter was measured 60 second after cuff release [29]. FMD was expressed as the percentage change in the brachial artery internal diameter from baseline following reactive hyperemia.

Carotid IMT was measured from the far wall of the right carotid artery within 10 mm proximal to the bifurcation on 2-dimensional ultrasound images. Three points were measured on 1 scan, which was synchronized with the R wave peaks on the ECG to avoid possible errors resulting from variable arterial compliance. Mean carotid IMT was calculated from 6 measurements on 2 scans [30,31].

To test the coefficient of repeatability of the CFR measurement, in 10 control subjects the measurement was repeated two days later. Intra observer intra-class correlation coefficient for coronary flow measurement was 0.847, and for CFR value it was 0.903. For FMD measurements, Intra observer intra-class correlation coefficient was 0.941. For IMT measurements, intra observer intra-class correlation coefficient was 0.967 [32].

Statistical Analyses

All analyses were conducted using SPSS 9.0 (SPSS for Windows 9.0, Chicago, IL). All group data are expressed as mean ± standard deviation. Considering the standard deviation of the control group measurements and accepting 10% change in CFR measurement as clinically significant, power analysis revealed that at least 37 subjects should have been included into the study. Continuous variables with normal distribution were compared using the *student t test* for multiple comparisons. Pearson's correlation analysis was used to test possible correlations. Variables with abnormal distribution and/or non continuous variables were analyzed via chi square analysis and spearman correlations test. A *P* value of < 0.05 was considered significant.

Results

Clinical Characteristics of the Study Population

The general characteristics and risk factors for coronary artery disease of the study population are presented in Table 1. Age, sex, BMI, heart rate, systolic blood pressure (BP), lipid profiles, and fasting glucose levels were similar between the groups. However, hemoglobin (12.9 ± 1.0 vs. 14.0 ± 1.1, P = 0.001), hematocrit (37.6 ± 2.4 vs. 42.1 ± 3.2 P < 0.001), were significantly lower in the BTM group compared to the controls. In the BTM group: HbA1 value was 93.4 ± 0.8 and HbA2 value was 5.6 ± 0.7. In the BTM group hsCRP value (3.8 ± 3.9 vs. 2.1 ± 1.6, P = 0.04) was significantly higher in the BTM group compared to the controls (Table 1).

Analyses of the Echocardiographic Measurements

Intraventricular septum (IVS) thickness, left ventricular posterior wall (PW) thickness, LVDD, LVSD, left ventricular ejection fraction (EF), left atrium diameter and LVMI were similar between the BTM group and the control group (Table 2).

Standard and Tissue Doppler Echocardiographic Analyses

Mitral E-wave was lower in the BTM group compared to the controls (71.0 ± 14.2 vs. 78.7 ± 14.5, P = 0.02), whereas, mitral A-wave was higher (68.0 ± 13.4 vs. 59.7 ± 11.6, P = 0.006).

Indicating left ventricular diastolic dysfunction E/A ratio was significantly lower in the BTM group (1.09 ± 0.27 vs. 1.34 ± 0.25, P < 0.001). Mitral E-wave deceleration time and isovolumic relaxation time were significantly higher in the BTM group as a manifestation of left ventricular diastolic dysfunction. Lateral *E*m did not differ between the groups, but lateral *A*m and lateral *E*m/*A*m ratio was significantly higher in BTM group than that in the control group (Table 2). E/Em ratio was lower in the BTM group as an implication of left ventricular diastolic dysfunction (3.79 ± 0.73 vs. 4.17 ± 0.95, P = 0.05) (Table 2).

Analysis of CFR Measurements

Baseline and peak heart rate and blood pressures were similar between the two groups. Accordingly rate-pressure product both at rest and after dipyridamole did not differ between the groups. Baseline diastolic peak flow velocity (DPFV) of LAD was significantly higher in the BTM group (23.8 ± 3.9 vs. 22.1 ± 3.0, P = 0.04). However, hyperemic DPFV was significantly lower (61.1 ± 13.0 vs. 68.2 ± 14.2, P = 0.02), and CFR was significantly lower (2.57 ± 0.46 vs. 3.07 ± 0.48, P < 0.0001) in the BTM group than that in the control group. Hyperemic DPFV acceleration time (AT) was similar between the two groups (52.9 ± 12.3 vs. 56.2 ± 12.4, P = 0.33). However, hyperemic DPFV deceleration time (DT) was significantly lower in BTM group (447.8 ± 149.9 vs. 550.4 ± 129.6, P = 0.003) (Table 3).

Table 1
Demographic and biochemical characteristics in patients with BTM and the control subjects.

	Patients with BTM (n = 40)	Healthy controls (n = 35)	P
Age (years)	37.8 ± 11.2	36.8 ± 5.2	0.59
Male/female (n/n)	19/21	17/18	0.79
Body-mass Index (kg/m^2)	26.1 ± 3.2	26.8 ± 2.1	0.20
Systolic BP (mmHg)	120.6 ± 11.3	120.0 ± 12.3	0.89
Diastolic BP (mmHg)	75.9 ± 7.4	76.3 ± 5.9	0.55
Heart rate (beats/min)	73.9 ± 7.1	72.7 ± 11.6	0.54
Total cholesterol (mg/dl)	175.6 ± 24.1	178.5 ± 30.6	0.59
HDL cholesterol (mg/dl)	46.3 ± 5.4	42.6 ± 9.5	0.06
LDL cholesterol (mg/dl)	106.2 ± 22.6	108.0 ± 22.9	0.73
Triglyceride (mg/dl)	109.2 ± 44.5	129.5 ± 63.6	0.12
hsCRP (mg/l)	3.8 ± 3.9	2.1 ± 1.6	0.09
Glucose (mg/dl)	93.6 ± 6.8	93.3 ± 5.8	0.31
Hemoglobin (g/dl)	12.9 ± 1.0	14.0 ± 1.1	<0.001
Hct (%)	37.6 ± 2.4	42.1 ± 3.2	<0.001
Ferritin (ng/ml)	131.8 ± 78.8	144.5 ± 44.7	0.46
RBC count (x10^{12}/l)	5.87 ± 0.46		
RDW (%)	15.83 ± 0.79		
MCV (fl)	61.11 ± 2.37		
HbA2 (%)	5.6 ± 0.7		
Mentzer Index	10.47 ± 1.07		
Green and King Index	45.89 ± 6.41		

Abbreviations: BP: blood pressure; HDL: high-density lipoprotein; LDL: low-density lipoprotein; RBC: red blood cell; MCV: mean cell volume; RDW: RBC distribution width; Mentzer Index: MCV/RBC; Green and King Index: MCVxMCVxRDW/(Hbx100); hsCRP: high-sensitivity C-reactive protein.

Table 2
Echocardiographic findings and standard and tissue Doppler parameters of the left ventricle.

	Patients with BTM (n = 40)	Healthy controls (n = 35)	P
IVS thickness (cm)	0.94 ± 0.13	0.91 ± 0.13	0.47
PW thickness (cm)	0.95 ± 0.12	0.90 ± 0.13	0.18
LVDD (cm)	4.63 ± 0.50	4.56 ± 0.42	0.54
LVSD (cm)	2.97 ± 0.33	2.86 ± 0.31	0.14
LAD (cm)	3.16 ± 0.37	3.04 ± 0.31	0.17
EF (%)	66.2 ± 4.0	67.0 ± 2.4	0.30
LVMI (g/m^2)	85.5 ± 16.8	79.6 ± 12.7	0.09
Mitral E-wave max (cm/s)	71.0 ± 14.4	78.7 ± 14.5	0.02
Mitral A-wave max (cm/s)	68.0 ± 13.4	59.7 ± 11.6	0.006
E/A ratio	1.09 ± 0.27	1.34 ± 0.25	<0.001
Mitral E-wave deceleration time (ms)	203.9 ± 33.7	187.2 ± 16.7	0.008
Izovolumic relaxation time (ms)	114.7 ± 13.9	97.5 ± 17.3	0.009
Lateral *E*m (cm/s)	19.2 ± 4.3	19.4 ± 3.5	0.93
Lateral *A*m (cm/s)	18.3 ± 3.7	14.2 ± 2.7	<0.001
Lateral *E*m/*A*m	1.09 ± 0.31	1.39 ± 0.32	<0.001
E/Em ratio	3.79 ± 0.73	4.17 ± 0.95	0.05

Abbreviations: IVS: interventricular septum; PW: posterior wall; LVDD: left ventricular diastolic diameter; LVSD: left ventricular systolic diameter; EF: ejection fraction; LVMI: left ventricular mass index; LAD: Left atrium diameter; *E*m: early peak velocity; *A*m: atrial peak velocity.

Brachial Artery FMD and Carotid IMT Measurements

Baseline and hyperemic brachial artery diameters were similar between the two groups (Table 3). Percent FMD measurements were significantly lower in the BTM group than that in the controls (6.22 ± 4.29 vs. 8.10 ± 4.00, P = 0.01). Carotid artery IMT measurements were significantly but slightly higher in the BTM group than that in the controls (56.5 ± 6.7 vs. 53.8 ± 4.2, P = 0.04). In BTM group 20% of the group had CFR lower than 2.5, but in control group only 2% had CFR lower than 2.5 P < 0.001. In BTM group 14% had percent FMD lower than 4.5, but in control group only 5% had percent FMD lower than 4.5 P = 0.04.

Table 3
Comparison of coronary flow reserve measurements.

	Patients with BTM (n = 40)	Healthy controls (n = 35)	P
Heart rate at rest (beats/min)	74.2 ± 6.4	73.0 ± 12.1	0.67
Systolic BP at rest (mmHg)	119.9 ± 11.6	120.0 ± 12.3	0.96
Diastolic BP at rest (mmHg)	75.7 ± 6.7	76.2 ± 5.9	0.67
Heart rate after dipyridamole (beats/min)	99.6 ± 10.7	97.6 ± 12.6	0.47
Systolic BP after dipyridamole (mmHg)	119.8 ± 10.5	119.2 ± 13.0	0.83
Diastolic BP after dipyridamole (mmHg)	74.2 ± 6.4	76.8 ± 5.8	0.07
Baseline DPFV (cm/s)	23.8 ± 3.9	22.1 ± 3.0	0.04
Baseline DPFV-AT(s)	52.6 ± 14.4	54.6 ± 18.2	0.66
Baseline DPFV-DT(s)	487.7 ± 185.3	590.1 ± 147.7	0.012
Hyperemic DPFV (cm/s)	61.1 ± 13.0	68.2 ± 14.2	0.02
Hyperemic DPFV-AT (s)	52.9 ± 12.3	56.2 ± 12.4	0.33
Hyperemic DPFV-DT (s)	447.8 ± 149.9	550.4 ± 129.6	0.003
CFR (ratio)	2.57 ± 0.46	3.07 ± 0.48	<0.0001
Basaline diameter (cm)	39.7 ± 7.1	41.4 ± 7.3	0.31
Hyperemic diameter (cm)	42.1 ± 7.4	44.8 ± 6.6	0.14
FMD percent	6.22 ± 4.29	8.10 ± 4.00	0.01
IMT (mm)	56.5 ± 6.7	53.8 ± 4.2	0.04
% With CFR < 2.5	20	2	<0.001
% With FMD percent < 4.5	14	5	0.04

Abbreviations: BP: blood pressure; RPP: rate-pressure product; DPFV: diastolic peak flow velocity; CFR: coronary flow reserve.

Relationship of CFR and Left Ventricular Diastolic Functions to Study Variables

CFR significantly and inversely correlated with hsCRP (r = -0,441, P < 0,001), Ferritin (r = −0.308, *P* = 0.02), (Table 4). Brachial artery FMD correlated slightly only with hsCRP (r = -0.241, P = .057). Carotid artery IMT correlated with hsCRP (r = 0.355, P = 0.003), ferritin (r = 0,375,P = 0,004), and glucose (r = 0.265, P = 0.024).

When the BTM group was divided into two as the mens and the womens groups, age was similar (38.3 ± 11.9 vs. 37.5 ± 6.1, P = 0.76) CFR values were similar (2.44 ± 0.42 vs 2.63 ± 0.51, P = 0.21), Brachial artery FMD values were similar (6.1 ± 4.7 vs. 4.8 ± 2.5, P = 0.32) between the two groups, whereas, carotid IMT values were slightly higher in mens group (0.59 ± 0.05 vs. 0.54 ± 0.07, P = 0.033), respectively. Both in mens and the womens groups hsCRP values correlated to CFR and IMT and FMD values (respectively r = -420, p value = 0.012; r = 0.462, p value = 0.005; r = -265, p value = 0.157 for men and r = -481, p value = 0.006; r = 0.330, p value = 0.069; r = -250, p value = 0.209 for women).

Discussion

In this study we have found that patients with BTM have significantly lower CFR compared to healthy subjects. Impairment of endothelial function and reduced CFR, which reflects coronary microvascular function, has been shown to be early manifestation of atherosclerosis and CAD [33,34]. Previous experimental and clinical studies have shown that early stage coronary atherosclerosis is frequently associated with abnormal resistance of the epicardial coronary arteries before segmental stenosis is apparent at coronary angiography [35,36]. Accordingly, diffuse atherosclerosis of the epicardial coronary arteries without segmental stenosis often causes reduced CFR, which may contribute to myocardial ischemia and abnormal perfusion during exercise or pharmacological vasodilatation [36]. Britten et al. [22] recently emphasized the prognostic importance of CFR with respect to atherosclerosis in subjects with normal coronary arteries or mildly diseased coronary arteries. In addition, Wang et al. [37] more recently showed that coronary vasoreactivity is reduced in asymptomatic adults with a greater coronary risk factor burden. Their findings corroborate that functional changes in coronary vasculature may occur in asymptomatic adults with risk factors, which supports reduced vasoreactivity as an indicator of CAD before its clinical manifestation. Our study is the first to implicate coronary microvascular dysfunction in patients with BTM. Hahalis

Table 4
Correlations between CFR, FMD, IMT and the other study parameters.

	Percent FMD	Carotid IMT	LVM	IVRT	hs-CRP	Ferritin	Glucose
CFR	r= ,480(**)	-,498(**)	-,007	-,254	-,441(**)	-,308(*)	-,130
	p=,000	,000	,956	,073	,000	,020	,275
Percent FMD		-,290(*)	,090	-,047	-,241	-,044	,043
		,017	,472	,745	,057	,748	,732
Carotid IMT			,047	,190	,355(**)	,375(**)	,265(*)
			,696	,187	,003	,004	,024
LVM				,330(*)	-,099	,217	-,014
				,020	,422	,108	,905
IVRT					-,002	-,007	-,020
					,988	,968	,894
hs-CRP						,290(*)	,126
						,032	,300
Ferritin							,131
							,330

Abbreviations: BP: blood pressure; RPP: rate-pressure product; DPFV: diastolic peak flow velocity; CFR: coronary flow reserve. IVS: interventricular septum; PW: posterior wall; LVDD: left ventricular diastolic diameter; LVSD: left ventricular systolic diameter; EF: ejection fraction; LVMI: left ventricular mass index; LAD: Left atrium diameter; *E*m: early peak velocity; *A*m: atrial peak velocity. BP: blood pressure; HDL: high-density lipoprotein; LDL: low-density lipoprotein; hsCRP: high-sensitivity C-reactive protein.
** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

G et al. [3] investigated the presence of endothelial dysfunction and subclinical atherosclerosis in non-transfusion-dependent patients with β-thalassemia intermedia (β-TI) by means of flow-mediated (FMD) and flow-independent (FID) brachial artery dilatation and carotid artery intima-media thickness. Their results indicated premature atherosclerosis among patients beta thalassemia intermedia [3]. Previous studies indicated increased carotid intima-media thickness and accelerated premature atherosclerosis in patients with beta thalassemia major [38,4], however, there have been no study on carotid intima media thickness in subjects with BTM. The present study is the first to indicate increased carotid IMT in subjects with BTM. Selek et al. [39] have suggested PON1 deficiency, an antioxidant enzyme with paraoxonase, arylesterase, and dyazoxonase activities, which contributes to the anti atherogenic effect of HDL and has preventive effect on oxidative modification of LDL. Therefore, decreased antioxidant status in subjects with BTM [39]. PON1 deficiency which means decreased antioxidant capacity is related to increased susceptibility to low density lipoprotein oxidation a well known cause of endothelial dysfunction, coronary microvascular impairment, and coronary atherosclerosis [40]. However, in patients with BTM little is known about the risk of atherogenesis [41]. In BTM subjects, the other study investigating oxidative status was the study of Vives Corrons et al. [42]. They found significantly increased oxidative stress compared to other forms of microcytic anemias including delta BTM and iron deficiency anemia [42].

In this study, measuring mitral E/A ratio, mitral E wave deceleration time, ısovolemic relaxation time, and E/Em ratio we have found that there is a statistically significant impairment in left ventricular diastolic function in subjects with BTM.

Our study implicated that subjects with BTM have increased carotid artery intima media thickness and decreased percent FMD of brachial artery. It is known that above mentioned findings are well established surrogates of developing atherosclerosis and vascular endothelial dysfunction.

Although low LDL-C level has been suggested to be associated with low risk of atherogenesis in thalassemia patients, it is well known that at any level of serum cholesterol, there is a wide variation in the incidence of coronary heart disease. Thus, beyond serum cholesterol levels, modification of LDL in the arterial wall, particularly by oxidation, is crucial to the cellular uptake of LDL in the first stages of atherosclerotic plaque development [43]. In our study we have registered subjects with normal LDL cholesterol levels for the BTM and the control groups. As PON1 inhibits oxidative modification of LDL-C, it can be suggested that BTM subjects may be more prone to development of atherogenesis than healthy subjects [39,40].

Our study implicated peripheral arterial endothelial dysfunction in subjects with BTM, whereas, our study has not conclusive implication to explain molecular basis for impairment in endothelial function in subjects with BTM. In our study we have found a negative significant correlation between hsCRP value and CFR, and a positive correlation between ferritin value and CFR. These parameters also correlated significantly to the carotid IMT value. Our study implicates that impaired coronary microvascular function in subjects with BTM have significant association to increased hsCRP value and decreased ferritin value. Previous studies have indicated significant association of hsCRP to CFR reflecting coronary microvascular function ant other surrogate markers of atherosclerosis [27,32,44]. In our study hsCRP values of BTM and the control groups were statistically comparable, however, in BTM group hsCRP values were slightly higher. Kanavaki et al. [45] have found increased endothelial activation and damage along with a state of chronic inflammation and increased hsCRP in beta-thalassemia intermedia. Our study has implicated association between CFR and hsCRP, however, our study does not shed light on molecular basis of increased hsCRP and its association to coronary microvascular function in BTM patients. In literature there is no study evaluating increased hsCRP and its molecular basis in BTM patients. Confirming these results we showed that CFR was impaired in patients with BTM and there was a strong inverse correlation between CFR and serum hsCRP levels, and a significant positive correlation in subjects with BTM. In this study, we might suggest that chronic inflammation represented by increased hsCRP might partly an explanation for early development of coronary microvascular and peripheral vascular endothelial dysfunction.

In conclusion, the present study demonstrated that CFR reflecting coronary microvascular function, and left ventricular diastolic function are impaired in patients with BTM. CFR and left ventricular diastolic function parameters well correlated with hsCRP and ferritin. Our results also indicate a strong significant relationship between left ventricular diastolic function and CFR. Although the number of patients included in this study is limited, these results suggest that impaired CFR may be an early manifestation of coronary vascular involvement in patients with BTM.

Study Limitations

Small sample size is the main limitation of our study which was caused partly by delicate inclusion and exclusion criteria. Our study represented unique preliminary data on vascular endothelial dysfunction in patients with BTM indicating necessity of larger scale studies on cardiovascular functions of patients with BTM.

Dyslipidaemia and hypertension are well established conditions impairing both CFR, FMD, and carotid IMT. Since we were unable to perform multivariate analysis due to small sample size, we excluded subjects with confounding factors for CFR and FMD measurements.

In this study, we have suggested early development of coronary microvascular and peripheral endothelial dysfunction, however, this study could not reveal exact results explaining molecular basis for early developing endothelial and microvascular dysfunction in BTM patients. We only suggest that increased hsCRP a representative of chronic inflammation, might partly be an explanation for earlier developing microvascular and endothelial dysfunction.

Conflict of Interest Statement

Each and every author does not have any personal or financial relationships that have any potential to inappropriately influence (bias) his or her actions or manuscript, and no financial or other potential conflicts of interest exist (includes involvement with any organization with a direct financial, intellectual, or other interest in the subject of the manuscript) regarding the manuscript. In addition, there are no any grants and sources of financial support related to the topic or topics of the manuscript.

References

[1] Sarnak MJ, Tighiouart H, Manjunath G, MacLeod B, Griffith J, Salem D, et al. Anemia as a risk factor for cardiovascular disease in The Atherosclerosis Risk in Communities (ARIC) study. J Am Coll Cardiol 2002;40:27–33.
[2] Brown DW, Giles WH, Croft JB. Hematocrit and the risk of coronary heart disease mortality. Am Heart J 2001;142:657–63.
[3] Hahalis G, Kalogeropoulos A, Terzis G, Tselepis AD, Kourakli A, Mylona P, et al. Premature atherosclerosis in non-transfusion-dependent β-thalassemia intermedia. Cardiology 2011;118(3):159–63.
[4] Stoyanova E, Trudel M, Felfly H, Lemsaddek W, Garcia D, Cloutier G. Vascularendothelial dysfunction in β-thalassemia occurs despite increased eNOS expression and preserved vascular smooth muscle cell reactivity to NO. PLoS One 2012;7(6):e38089.
[5] Eldor A, Rachmilewitz EA. The hypercoagulable state in thalassemia. Blood 2002;99:36–43.
[6] Praticó D, Pasin M, Barry OP, Ghiselli A, Sabatino G, Iuliano L, et al. Iron-dependent human platelet activation and hydroxyl radical formation: involvement of protein kinase C. Circulation 1999;99:3118–24.
[7] Balla G, Jacob HS, Eaton JW, Belcher JD, Vercellotti GM. Hemin: a possible physiological mediator of low density lipoprotein oxidation and endothelial injury. Arterioscler Thromb 1991;11:1700–11.
[8] Kunsch C, Medford RM. Oxidative stress as a regulator of gene expression in the vasculature. Circ Res 1999;85:753–66.
[9] Schechter AN, Gladwin MT. Hemoglobin and the paracrine and endocrine functions of nitric oxide. N Engl J Med 2003;348:1483–5.

[10] Heitzer T, Schlinzig T, Krohn K, Meinertz T, Münzel T. Endothelial dysfunction, oxidative stress, and risk of cardiovascular events in patients with coronary artery disease. Circulation 2001;104:2673–8.
[11] Crowley JP, Sheth S, Capone RJ, Schilling RF. A paucity of thalassemia trait in Italian men with myocardial infarction. Acta Haematol 1987;78:249–51.
[12] Wang CH, Schilling RF. Myocardial infarction and thalassemia trait: an example of heterozygote advantage. Am J Hematol 1995;49:73–5.
[13] Gallerani M, Scapoli C, Cicognani I, Ricci A, Martinelli L, Cappato R, et al. Thalassaemia trait and myocardial infarction: low infarction incidence in male subjects confirmed. J Intern Med 1991;230:109–11.
[14] Namazi MR. Minor thalassemia as a protective factor against cerebrovascular accidents. Med Hypotheses 2002;59:361–2.
[15] Tesoriere L, D'Arpa D, Maggio A, Giaccone V, Pedone E, Livrea MA. Oxidation resistance of LDL is correlated with vitamin E status in beta-thalassemia intermedia. Atherosclerosis 1998;137:429–35.
[16] Maioli M, Pettinato S, Cherchi GM, Giraudi D, Pacifico A, Pupita G, et al. Plasma lipids in beta-thalassemia minor. Atherosclerosis 1989;75:245–8.
[17] Burch GE, de Pasquale NP. Hematocrit, blood viscosity and myocardial infraction. Am J Med 1962;32:161–3.
[18] Vigi V, Volpato S, Gaburro D, Conconi F, Bargellesi A, Pontremoli S. The correlation between red-cell survival and excess of alpha-globin synthesis in beta-thalassemia. Br J Haematol 1969;16:25–30.
[19] Mancini GB, Dahlöf B, Díez J. Surrogate markers for cardiovascular disease: structural markers. Circulation 2004;109:22–30.
[20] Levenson J, Gariepy J, Del-Pino M, Salomon J, Denarie N, Simon A. Association of plasma viscosity and carotid thickening in a French working cohort. Am J Hypertens 2000;13:753–8.
[21] Drüeke T, Witko-Sarsat V, Massy Z, Descamps-Latscha B, Guerin AP, Marchais SJ, et al. Iron therapy, advanced oxidation protein products, and carotid artery intima-media thickness in end-stage renal disease. Circulation 2002;106:2212–7.
[22] Britten MB, Zeiher AM, Schachinger V. Microvascular dysfunction in angiographically normal or mildly diseased coronary arteries predicts adverse cardiovascular long-term outcome. Coron Artery Dis 2004;15:259–64.
[23] Goel PK, Gupta SK, Agarwal A, Kapoor A. Slow coronary flow: a distinct angiographic subgroup in syndrome X. Angiology 2001;52:507–14.
[24] Beltrame JF, Limaye SB, Horowitz JD. The coronary slow flow phenomenon - a new coronary microvascular disorder. Cardiology 2002;97:197–202.
[25] Machado Cesar LA. Coronary slow flow phenomenon: revisiting a concept not so new. Cardiology 2003;100:47.
[26] Okan V, Cigiloglu A, Cifci S, Yilmaz M, Pehlivan M. Red cell indices and functions differentiating patients with the beta-thalassaemia trait from those with iron deficiency anaemia. J Int Med Res 2009;37:25–30.
[27] Caliskan M, Erdogan D, Gullu H, Yilmaz S, Gursoy Y, Yildirir A, et al. Impaired coronary microvascular and left ventricular diastolic functions in patients with ankylosing spondylitis. Atherosclerosis 2008;196:306–12.
[28] Erdogan D, Gullu H, Caliskan M, Yildirim I, Ulus T, Bilgi M, et al. Coronary flow reserve and coronary microvascular functions are strongly related to serum uric acid concentrations in healthy adults.Coron Artery Dis 2006;17:7–14 [12].
[29] Coretti MC, Anderson TJ, Benjamin EJ, et al. Guideline for the ultrasound assessment of endothelium-dependent flow-mediated vasodilatation of the brachial artery. J Am Coll Cardiol 2002;39:257–62.
[30] Crouse III JR, Craven TE, Hagaman AP, Bond MG. Association of coronary disease with segment-spesific intimal-medial thickening of the extracranial carotid artery. Circulation 1995;92:1141–7.
[31] Ciccone MM, Scicchitano P, Zito A, Agati L, Gesualdo M, Mandolesi S, et al. Correlation between coronary artery disease severity, left ventricular mass index and carotid intima media thickness, assessed by radio-frequency. Cardiovasc Ultrasound Nov 16 2011;9:32.
[32] Gullu H, Erdogan D, Caliskan M, Tok D, Yildirim E, Ulus T, et al. Interrelationship between noninvasive predictors of atherosclerosis: transthoracic coronary flow reserve, flow-mediated dilation, carotid intima-media thickness, aortic stiffness, aortic distensibility, elastic modulus, and brachial artery diameter. Echocardiography 2006;23:835–42.
[33] Caiati C, Zedda N, Montaldo C, Montischi R, Iliceto S. Contrast-enhanced transthoracic second harmonic echo Doppler with adenosine: a noninvasive, rapid and effective method for coronary flow reserve assessment. J Am Coll Cardiol 1999;34:122–30.
[34] Lambertz H, Tries HP, Stein T, Lethen H. Noninvasive assessment of coronary flow reserve with transthoracic signal-enhanced Doppler echocardiography. J Am Soc Echocardiogr 1999;12:186–95.
[35] Wikstrom J, Gronros J, Bergstrom G, Gan LM. Functional and morphologic imaging of coronary atherosclerosis in living mice using high-resolution color Doppler echocardiography and ultrasound biomicroscopy. J Am Coll Cardiol 2005;46:720–7.
[36] De Bruyne B, Hersbach F, Pijls NH, Bartunek J, Bech JW, Heyndrickx GR, et al. Abnormal epicardial coronary resistance in patients with diffuse atherosclerosis but "Normal" coronary angiography. Circulation 2001;104:2401–6.
[37] Wang L, Jerosch-Herold M, Jacobs Jr DR, Shahar E, Folsom AR. Coronary risk factors and myocardial perfusion in asymptomatic adults: the Multi-Ethnic Study of Atherosclerosis (MESA). J Am Coll Cardiol 2006;47:565–72.
[38] Hahalis G, Kremastinos DT, Terzis G, Kalogeropoulos AP, Chrysanthopoulou A, Karakantza M, et al. Global vasomotor dysfunction and accelerated vascular aging in beta-thalassemia major. Atherosclerosis 2008;198:448–57.
[39] Selek S, Aslan M, Horoz M, Gur M, Erel O. Oxidative status and serum PON1 activity in beta-thalassemia minor. Clin Biochem 2007;40:287–91.
[40] Canales A, Sanchez-Muniz FJ. Paraoxanase, something more than an enzyme? Med Clin (Barc) 2003;121:537–48.
[41] Tassiopoulos S, Deftereos S, Konstantopoulos K, Farmakis D, Tsironi M, Kyriakidis M, et al. Does heterozygous beta-thalassemia confer a protection against coronary artery disease? Ann N Y Acad Sci 2005;1054:467–70.
[42] Vives Corrons JL, Miguel-Garcia A, Pujades MA, Miguel-Sosa A, Cambiazzo S, Linares M, et al. Increased susceptibility of microcytic red blood cells to in vitro oxidative stress. Eur J Haematol 1995;55:327–31.
[43] Steinberg D, Lewis A. Conner Memorial Lecture. Oxidative modification of LDL and atherogenesis. Circulation 1997;95:1062–71.
[44] Vaccarino V, Khan D, Votaw J, Faber T, Veledar E, Jones DP, et al. Inflammation is related to coronary flow reserve detected by positron emission tomography in asymptomatic male twins. J Am Coll Cardiol Mar 15 2011;57(11):1271–9.
[45] Kanavaki I, Makrythanasis P, Lazaropoulou C, Tsironi M, Kattamis A, Rombos I, et al. Soluble endothelial adhesion molecules and inflammation markers in patients with beta-thalassemia intermedia. Blood Cells Mol Dis Nov-Dec 2009;43(3):230–4.