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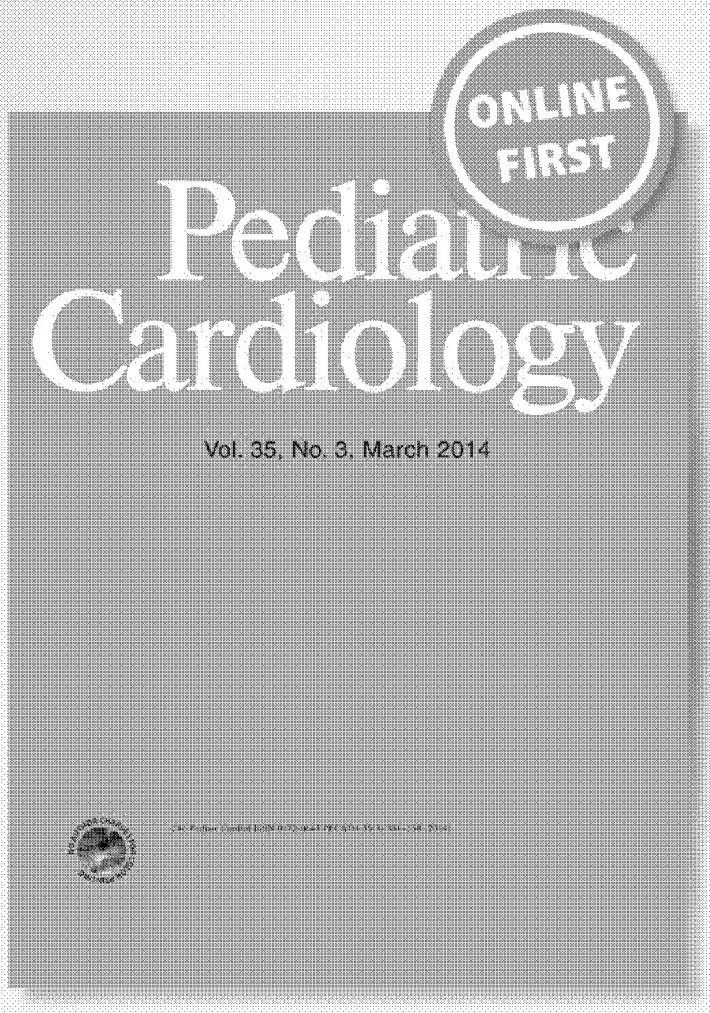

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ORIGINAL ARTICLE

Subclinical Vascular Endothelial Dysfunctions and Myocardial Changes With Type 1 Diabetes Mellitus in Children and Adolescents

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Abstract Vascular endothelial dysfunction, accelerated thickening of arterial intima, and changes in ventricular functions contribute to increased cardiovascular morbidity in type 1 diabetes mellitus (T1DM). This study aimed to investigate the functional-structural changes in the arteries and myocardium together with affection of highly sensitive C-reactive protein (hsCRP), circulating endothelial cells (CECs), and vitamin C levels in children with T1DM. Also, to test the association with early atherosclerotic changes. The study included 30 children with a diagnosis of T1DM and 30 healthy subjects matched by sex, age, and body mass index. Serum lipids, HbA1c, hsCRP, vitamin C, and CECs were detected. Corrected QT interval (QTc), cardiac dimensions, and left ventricular (LV) functions were assessed using conventional echocardiography. Noninvasive ultrasound was used to measure brachial artery flow-mediated dilation (FMD) responses and carotid intima-media thickness (IMT). The QTc interval was significantly higher in the diabetic patients than in the control subjects ($P < 0.001$). The findings showed LV diastolic dysfunction as reflected by significantly lower early peak flow velocity, decreased *E*/*A* ratio, increased early filling deceleration time (DcT), and prolonged isovolumic relaxation time (IVRT) ($P < 0.001$ for each). The children with diabetes had a significantly lower FMD response, increased IMT, lower vitamin C level, higher hsCRP, and higher CEC compared with the control subjects ($P < 0.001$ for each). A positive correlation between CEC and HbA1c was found ($P = 0.004$). An alteration in myocardial function and endothelial dysfunction may begin early with the association of early atherosclerotic changes. These changes are accelerated when glycemic control is poor. The authors recommend early and close observation of children with diabetes for any alterations in cardiac and vascular endothelial function. Vitamin C supplementation may reduce the risk of complications.

Keywords Children · Diabetes mellitus · Endothelial dysfunction · Ventricular dysfunctions · Inflammatory markers

Type 1 diabetes mellitus (T1DM) is an important risk factor for cardiovascular events [25]. Individuals with diabetes have a 2- to 10-fold increased risk for the development of atherosclerotic diseases compared with the normal population [8]. Thus, increased morbidity and mortality due to coronary, cerebrovascular, and peripheral arterial disease have been observed [12]. Even if these complications become manifest only in the adult diabetic patient, the process of vascular changes starts much earlier [22, 45].

Atherosclerosis in young adults is associated with the prediabetic state. As shown in autopsies, the atherosclerotic processes at the endothelial level begin in childhood and progress rapidly in the presence of risk factors [5, 23].

The diabetic state is characterized by alterations in serum lipoproteins that may enhance their susceptibility to oxidation [11]. It has been suggested that the increased risk

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of atherosclerotic disease in individuals with diabetes may be due to enhanced foam cell formation after greater susceptibility of low-density lipoprotein (LDL) to oxidation [11, 35].

Endothelial dysfunction plays a key role in the pathogenesis of diabetic vascular disease, which is the principal cause of mortality and morbidity in diabetic patients [1, 49]. However, hyperglycemia itself can cause endothelial injury and dysfunction through several mechanisms [26]. Exposure of endothelial cells to high glucose levels increases oxidative stress. This causes enhanced generation of reactive oxygen species and reduction of antioxidant reserves. Hyperglycemia also increases the production of advanced glycation end products as well as the activation of the protein kinase C and polyol pathways [32]. These hyperglycemia-mediated mechanisms disrupt cell cycling, which in turn causes inhibition of cell proliferation and migration as well as an increase in the apoptosis of endothelial cells [61].

In recent years, circulating endothelial cells (CECs) have emerged as markers of vascular damage. Very small numbers of these cells exist in healthy individuals, but their numbers increase dramatically in diseases with vascular damage such as cardiovascular disease, specific infections, vasculitis, and type 2 diabetes [30, 38]. Evidence exists to show that metabolic disturbances, myocardial fibrosis, small vessel disease, cardiac autonomic neuropathy, and insulin resistance all may contribute to the development of diabetic cardiomyopathy [13, 29].

Changes in vascular function and structure appear to predict an increased risk of cardiovascular morbidity and mortality later in life [40, 48, 54]. Enhanced oxidative stress, a central mechanism of vascular complications in T1DM, also is being evoked as a pathogenic link to prolongation of the QTc interval [34, 39].

High-resolution ultrasound is a reliable, noninvasive method for detecting early structural and functional atherosclerotic changes in the arterial wall. Increased carotid intima-media thickness (IMT), a structural marker of early atherosclerosis, relates to the severity and extent of coronary artery disease and predicts the likelihood of cardiovascular events in population groups. Flow-mediated dilation (FMD) of the brachial artery is a marker of endothelial function that can be assessed by measuring arterial diameter responses to increased flow [51].

Conventional echocardiography using standardized protocol with M-mode, two-dimensional, pulsed-wave, continuous-wave, and color-flow Doppler capabilities can be used for the diagnosis of diabetic cardiomyopathy or diabetes-induced myocardial dysfunction [31].

This study aimed to investigate the functional-structural changes of the arteries and myocardium in asymptomatic children and adolescents with T1DM together with affection of the biomarker of inflammation (hsCRP), CECs, and vitamin C levels. The hypothesis that T1DM can be associated with early atherosclerotic changes also was tested.

Methods

Subjects

This case–control study investigated 30 children (17 males and 13 females) ages 5–16 years with a diagnosis of T1DM. Another 30 apparently healthy children matched by age, sex, and body mass index (BMI) were enrolled in the study as the control group. Children with diabetes were recruited from the diabetic outpatient clinic of the Pediatric Department of Assiut Children's University Hospital during the period May 2012 to April 2013.

The criteria for inclusion in the study specified children with a diagnosis of T1DM who had no symptoms or complications including renal impairment, retinopathy, neuropathy, and clinical symptoms of cardiac dysfunction or cardiomyopathy; who had a 1- to 4-year history of diabetes, and who were receiving no medications other than daily insulin. The exclusion criteria ruled out children with T1DM complications including renal impairment, retinopathy, neuropathy, cardiac dysfunction, and other associated systemic diseases or chronic illness, as well as those whose diabetes had a duration longer than 4 years.

The healthy control children included in the study were volunteers in Assiut Children's University Hospital. After obtaining approval from the medical ethical committee of the Faculty of Medicine at Assiut University, an informed written consent was taken from the parents or legal guardians of the study children.

All the patients were subjected to a full history-taking that included age, duration of illness, family history of diabetes, and frequency of diabetic ketoacidosis, as well as a thorough clinical examination with special emphasis on weight, height, BMI, and systolic and diastolic blood pressure. The investigations performed for all the study participants included serum lipogram and HBA1c, plasma levels of vitamin C and highly sensitive C-reactive protein (hsCRP), level of CECs, electrocardiography (ECG), echocardiography, and vascular ultrasound studies for measurement of carotid IMT.

Electrocardiograaphy (ECG)

All the patients included in the study underwent 12-lead resting ECG. The heart rate-corrected QT (QTc) interval was calculated by the use of Bazett's formula and expressed in seconds [4].

Echocardiography

An echocardiography was performed using the Philips EnVisor C HD ultrasound system (Philips Medical Systems, Inc., Netherlands) with a S4-2 Broadband Sector (4- to 2-MHz phased-array transducer). An M-mode echocardiography was obtained on the left sternal border. Left ventricular (LV) dimension, LV fractional shortening (FS), and LV ejection fraction (EF) were measured [41].

LV diastolic function was evaluated by mitral inflow velocities obtained in the apical four-chamber view. The early peak flow velocity (*E*) and the atrial filling velocity (*A*) were measured, and the *E/A* ratio was calculated. The interval from the early peak velocity to the zero intercept of the extrapolated deceleration slope (early filling deceleration time [DcT]) was measured. The interval between the end of the LV outflow velocity and the onset of mitral inflow (isovolumic relaxation time [IVRT]), obtained by pulsed-wave Doppler with the cursor placed in the LV outflow near the anterior leaflet of the mitral valve, was measured from the end of the LV ejection to the onset of the mitral inflow [41].

Vascular Ultrasound Studies

All studies were performed using the Philips EnVisor C HD ultrasound system (Philips Medical Systems) with an L12-3+ Broadband Linear (12- to 3-MHz linear-array transducer) [33].

Brachial Artery Physiology

The brachial artery was scanned above the antecubital fossa, and its diameter was measured from B-mode ultrasound images with the patient at rest (baseline brachial artery diameter). Reactive hyperemia then was induced by inflation of a pneumatic tourniquet placed around the forearm to a pressure of 250 mmHg for 4.5 min, followed by release. A second scan of vessel diameter was performed at a fixed distance manually using ultrasonic calipers (maximal brachial artery diameter). Longitudinal images were scanned and captured (in millimeters) at end-diastole incident with the R-wave on a continuously recorded ECG. The FMD was calculated and expressed as a percentage [24, 33].

Measurement of Carotid IMT

All studies were conducted according to a predetermined, standardized scanning protocol for the right and left carotid arteries [22]. The proximal part of the carotid bulb was identified on both sides, and the segments of the common carotid arteries 1–2 cm proximal to the bulb were scanned [28]. The image was focused on the posterior (far) wall, and the resolution box function was used to magnify the arterial far wall. Two angles (anterior oblique and lateral) were used in each case for common carotid IMT on both sides. One end-diastolic frame (captured adjacent to the R-wave on a continuously recorded ECG) for each interrogation angle was selected and analyzed for mean and maximum IMT using ultrasound calipers [23]. The images were analyzed by independent trained readers blinded to the subject's clinical details.

Biochemical Studies

Serum Lipids and Blood HbA1c

Venous blood samples were taken in the morning after an overnight fast (10–12 h). Serum total cholesterol, high-density lipoprotein (HDL), and triglyceride concentrations were measured by standard enzymatic methods using Boehringer Mannheim GmbH (Germany) reagents with a fully automated analyzer. Calculation of LDL concentration was performed using Friedewald's equation [14]. High-performance liquid chromatography (Variant Analyzer; Bio-Rad, Inc., Cairo, Egypt) was used to measure HbA1c.

Plasma Vitamin C

The plasma concentration of vitamin C was measured by the colorimetric method based on redox reaction of ascorbate with 2,6-dichlorophenol indophenolin acid solution involving reduction of dye to a colorless leucobase while ascorbate was oxidized to dehydroascorbate (DHA). With this method, absorbance was measured at 520 nm using the kit manufactured by Biodiagnostic, Inc., Cairo, Egypt.

Plasma hsCRP

The plasma level of hsCRP was measured using the High-Sensitivity C-Reactive Protein (hsCRP) Enzyme Immunoassay Test (ELISA) kit for quantitative determination of the C-reactive protein concentration in human serum (catalog no. E29-056; Immunospec Corp., Canoga Park, CA, USA).

Isolation and Measurement of CECs

To isolate and enumerate CECs, we used the modified study protocol described by Woywodt et al. [59]. This technique depends on the use of paramagnetic particles coated with antibodies directed against the CD146 molecule found on endothelial cells.

Blood was obtained by nontraumatic venipuncture and collected in two 7.5-mL ethylenediaminetetraacetic acid

Table 1 Characteristics of the study groups

	Diabetic children ($n = 30$)	Control children ($n = 30$)	P value
Age (years)	11.1 ± 3.8	9.8 ± 3.5	0.241
Weight (kg)	44.8 ± 12.3	46.6 ± 12.4	0.579
Height (m)	1.51 ± 0.09	1.52 ± 0.12	0.791
BMI (kg/m^2)	19.2 ± 2.4	20.1 ± 3.8	0.241
Duration of DM (years)	3.92 ± 0.62	–	–
Positive family history	10/30	–	–
DKA	13/30	–	–
Systolic blood pressure (mmHg)	112 ± ^{2}88	111 ± 9	0.139
Diastolic blood pressure (mmHg)	65 ± 7	66 ± 7	0.458
HbA1c (%)	9.7 ± 2.2	4.9 ± 0.4	<0.001**
Total cholesterol (mmol/L)	154.7 ± 29.2	132 ± 12.4	0.003*
HDL (mmol/L)	60.5 ± 28.8	29.3 ± 3.5	<0.001**
LDL (mmol/L)	110.4 ± 28.1	99.1 ± 1.5	0.089
Triglycerides (mmol/L)	97.7 ± 50.7	66.5 ± 4.9	0.022*
Vitamin C (μmol/L)	3.5 ± 0.2	5.1 ± 0.3	<0.001**
hsCRP (mg/L)	329 ± 20.5	154.9 ± 16.8	<0.001**

Continuous variables are expressed as the mean ± standard deviation

BMI body mass index, *DM* diabetes mellitus, *DKA* diabetic ketoacidosis, *HbA1c* glycosylated hemoglobin, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *hsCRP* high-sensitivity C-reactive protein

*$P < 0.05$; **$P < 0.001$

tubes. The first tube was discarded to avoid false-positive results caused by dislodging of endothelial cells during venipuncture. Next, 1 mL of blood from the second tube was diluted in 1 mL of bovine serum albumin (BSA)/phosphate-buffered saline (PBS) buffer (0.1 % BSA in PBS) and blocked by 20 mL of Fc receptor (Miltenyi, Gladbach, Germany) for 10 min at 4 °C. Then, 50 mL of anti-CD146-coated M-450 paramagnetic particles (Dynabeads, Inc., Dynal, Norway) were added and mixed thoroughly.

The sample was mixed in a head-over-head mixer for 30 min at 4 °C and washed four times with PBS/BSA buffer in front of a magnet (Dynal, MPC-L, Dynal, Norway). These isolated cells then were further stained with a specific endothelial cell marker. Next, 50 μL of a 2-mg/mL fluorescein isothiocyanate conjugated (FITC)-coupled UEA-1 solution (Sigma-Aldrich, Inc., St. Louis, MO, USA) was added, and the sample was incubated for half an hour in darkness, then washed twice and suspended in 200 mL of PBS solution. Cells were counted by flow cytometry (FACSCaliber, BD, USA). The number of CECs was expressed as cells per mL of blood [59].

Table 2 Echocardiographic parameters derived from the two-dimensional and Doppler imaging in the study groups

Parameter	Diabetic children ($n = 30$)	Control children ($n = 30$)	P value
LVEDD (mm)	40.59 ± 4.28	41.19 ± 4.23	0.59
LVESD (mm)	24.56 ± 3.68	25.01 ± 3.91	0.66
IVSd (mm)	7.35 ± 0.78	7.41 ± 0.53	0.73
LVPWd (mm)	4.08 ± 0.56	4.09 ± 0.54	0.97
EF (%)	70.53 ± 4.45	70.10 ± 4.65	0.72
FS (%)	39.70 ± 3.68	39.48 ± 3.93	0.83
E (cm/s)	84.51 ± 6.29	111.61 ± 15.00	<0.001**
A (cm/s)	59.13 ± 8.38	61.66 ± 11.20	0.33
E/*A* ratio	1.45 ± 0.19	1.85 ± 0.34	<0.001*
DcT (ms)	198.99 ± 15.24	119.97 ± 14.62	<0.001**
IVRT (ms)	86.66 ± 8.77	57.26 ± 6.47	<0.001**

All measures are expressed as the mean ± standard deviation

LVEDD left ventricular end-diastolic diameter, *LVESD* left ventricular end-systolic diameter, *IVSd* diastolic interventricular septal thickness, *LVPWd* diastolic posterior wall thickness, *EF* ejection fraction, *FS* fractional shortening, *E* mitral E velocity, *A* mitral A velocity, *DcT* mitral deceleration time, *IVRT* isovolumic relaxation time

*$P < 0.05$; **$P < 0.001$

Statistical Analysis

Data analysis was performed using the Statistical Package for the Social Sciences, version 16 (SPSS, Inc., Chicago, IL, USA). Descriptive statistics were calculated. Results are expressed as mean ± standard deviation and percentage. Cross tabulation was performed using chi-square for categorical data. Comparisons between the two groups (diabetic patients and control subjects) were performed using Student's t test. Multivariate correlation analyses were performed by the linear regression technique. All P values lower than 0.05 were considered statistically significant.

Results

The characteristics of the study groups are shown in Table 1. The groups did not differ significantly in terms of age, gender distribution, body size, or blood pressure. As shown in Table 1, the diabetic patients had higher levels of HbA1c ($P = 0.003$), total serum cholesterol ($P < 0.001$), HDL cholesterol ($P = 0.022$), serum triglycerides ($P < 0.001$), and hsCRP ($P < 0.001$) than the control patients. Whereas the LDL cholesterol levels were not significantly different between the cases and control subjects, the plasma level of vitamin C was significantly lower

Table 3 Vascular ultrasound parameters in the study groups

Parameter	Diabetic children ($n = 30$)	Control children ($n = 30$)	P value
Brachial artery baseline diameter (mm)	3.12 ± 0.35	3.11 ± 0.34	0.92
Peak FMD (%)	4.60 ± 2.13	9.31 ± 2.29	<0.001**
Maximum carotid IMT (mm)	0.55 ± 0.04	0.50 ± 0.02	<0.001**
Mean carotid IMT (mm)	0.48 ± 0.02	0.41 ± 0.02	<0.001**

All measures are expressed as the mean ± standard deviation

FMD flow-mediated dilation, *IMT* intima-media thickness

*$P < 0.05$; **$P < 0.001$

Table 4 Correlation between QTc and subject characteristics

QTc	Pearson correlation P value	R
Vitamin C	<0.001**	−0.752
hsCRP	<0.001**	0.851
CEC count	0.007**	0.426
E velocity	<0.001**	−0.677
E/A ratio	<0.001**	−0.576
IVRT	<0.001**	0.733
DcT	<0.001**	0.676
Peak FMD	<0.001**	−0.627
Mean carotid IMT	<0.001**	0.766

hsCRP high-sensitivity C-reactive protein, *CEC* circulating endothelial cell, *E* mitral E velocity, *A* mitral A velocity, *IVRT* isovolumic relaxation time, *DcT* mitral deceleration time, *FMD* flow-mediated dilation, *IMT* intima-media thickness

*$P < 0.05$; **$P < 0.001$

in the diabetic patients than in the control subjects ($P < 0.001$).

The data derived from conventional echocardiographic analysis are listed in Table 2. The two groups did not differ significantly in terms of LV dimensions or systolic function. However, the patients with T1DM demonstrated discrete impairment of the diastolic function, as reflected by decreased *E*-wave velocity and *E/A* ratio ($P < 0.001$) and increased DcT and IVRT ($P < 0.001$).

Table 3 represents the results of vascular ultrasound studies. Peak FMD was significantly lower in the children with diabetes than in the control subjects ($P < 0.001$), whereas the children with diabetes had a significantly greater carotid IMT than the control children ($P < 0.001$). The two groups did not differ significantly in terms of brachial artery baseline diameter. The duration of the QT interval corrected for heart rate (QTc sec.) was found to be significantly longer in the children with T1DM than in the control group (0.467 ± 0.184 vs 0.414 ± 0.174 s; $P < 0.001$), representing an abnormal prolongation of ventricular repolarization among diabetic children.

The numbers of CECs increased significantly in the diabetic children relative to the control children (102.3 ± 15.4 vs 36.3 ± 5.1 cells/mL; $P < 0.001$).

Table 4 summarizes the Pearson rank correlation between the QT interval corrected for heart rate (QTc) and the clinical, biochemical, echocardiographic, and vascular ultrasound parameters among the T1DM children. We noted a negative correlation between the QTc and the plasma concentration of vitamin C ($r = -0.752$; $P < 0.001$), the *E* velocity ($r = -0.677$; $P < 0.001$), and the *E/A* ratio ($r = -0.576$; $P < 0.001$). A positive correlation was seen between QTc and the plasma level of hsCRP ($r = 0.851$; $P < 0.001$), CEC count ($r = 0.426$; $P = 0.007$), IVRT ($r = 0.733$; $P < 0.001$), and DcT ($r = 0.676$; $P < 0.001$). We found QTc negatively correlated with peak FMD ($r = -0.627$; $P < 0.001$) and positively correlated with mean carotid IMT ($r = 0.766$; $P < 0.001$).

Figure 1 shows a negative correlation between the peak FMD and the mean carotid IMT ($r = -0.624$; $P < 0.001$), and Fig. 2 shows a negative correlation between the plasma concentration of vitamin C and the mean carotid IMT ($r = -0.701$; $P < 0.001$). Figure 3 shows a positive correlation between the CEC count and HbA1c ($r = 0.516$; $P = 0.004$).

Discussion

The mechanisms of endothelial dysfunction and accelerated atherosclerosis in diabetes are multifactorial and have not been fully characterized. The pathogenesis of atherosclerosis is importantly related to high levels of total cholesterol and LDL cholesterol and low levels of HDL cholesterol [42]. Interestingly, the current study showed a trend toward significantly increased total cholesterol and HDL cholesterol levels in children with T1DM. These data are in line with prior reports [3, 27, 52, 53].

Although higher levels of plasma HDL cholesterol are widely thought to be atheroprotective, in the setting of T1DM, HDL cholesterol may be dysfunctional in combating the adverse, proinflammatory, and proatherogenic effects of oxidized LDL cholesterol. The presence of dysfunctional HDL cholesterol would make T1DM subjects more vulnerable to oxidative vascular damage despite higher absolute levels [43].

Under normal physiologic conditions, a critical balance exists in the generation of oxygen free radicals and antioxidant defense systems used by organisms to deactivate and protect themselves against free radical toxicity [19,

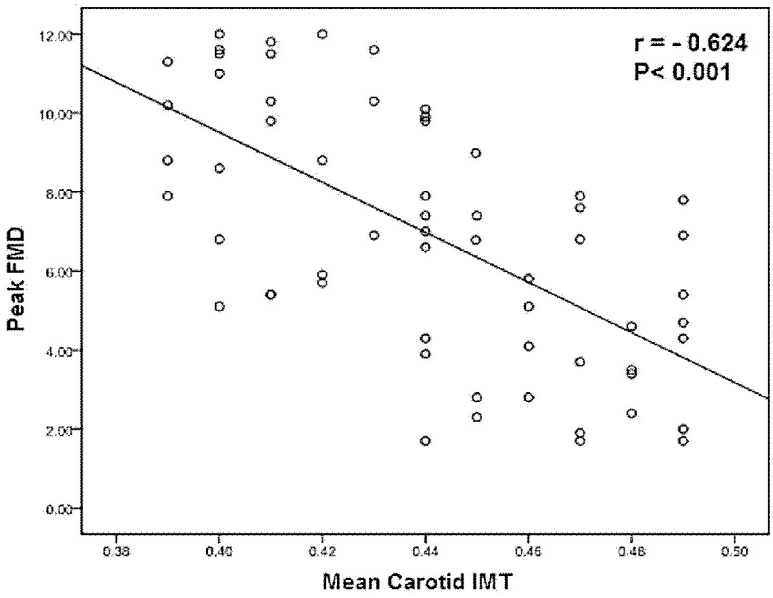

Fig. 1 Correlation coefficient showing the negative relation between peak flow-mediated dilation (FMD) and mean carotid intima-media thickness (IMT)

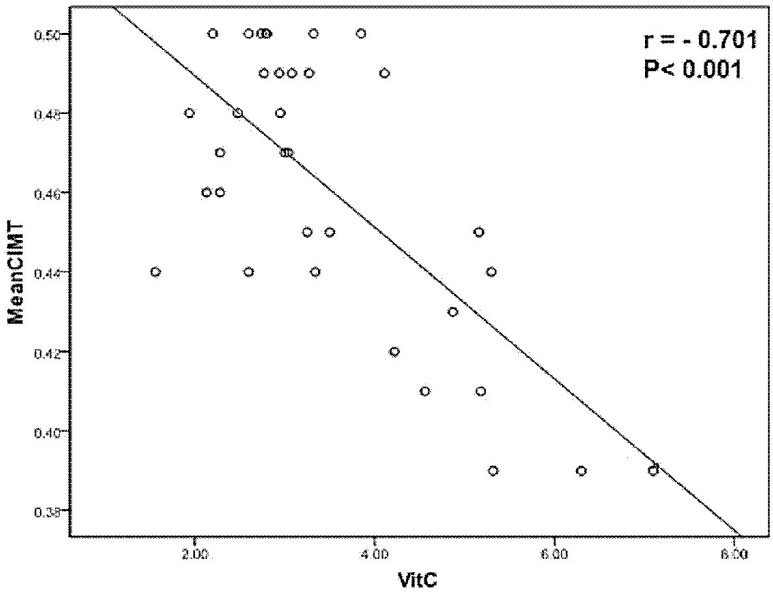

Fig. 2 Correlation coefficient showing the negative relation between plasma concentration of vitamin C and mean carotid intima-media thickness (IMT)

50]. Impairment in the oxidant/antioxidant equilibrium creates a condition known as oxidative stress. Oxidative stress is known to be a component of molecular and cellular tissue damage mechanisms in a wide spectrum of human diseases [7, 18]. The mechanisms producing the increased oxidative stress in diabetes include not only oxygen free radical generation due to nonenzymatic glycosylation (glycation) and autooxidation of glycation products but also changes in the tissue content and activity of antioxidant defense systems.

One of the major natural antioxidants derived from natural sources by dietary intake is vitamin C [36]. The current study demonstrated a significantly lower plasma concentration of vitamin C in the diabetic children than in the control children. These results are in accordance with those of previous studies [46, 57], which showed that children with T1DM have low levels of the antioxidant vitamin C.

hsCRP is an acute-phase protein associated with systemic inflammation. In this study, the circulating levels of

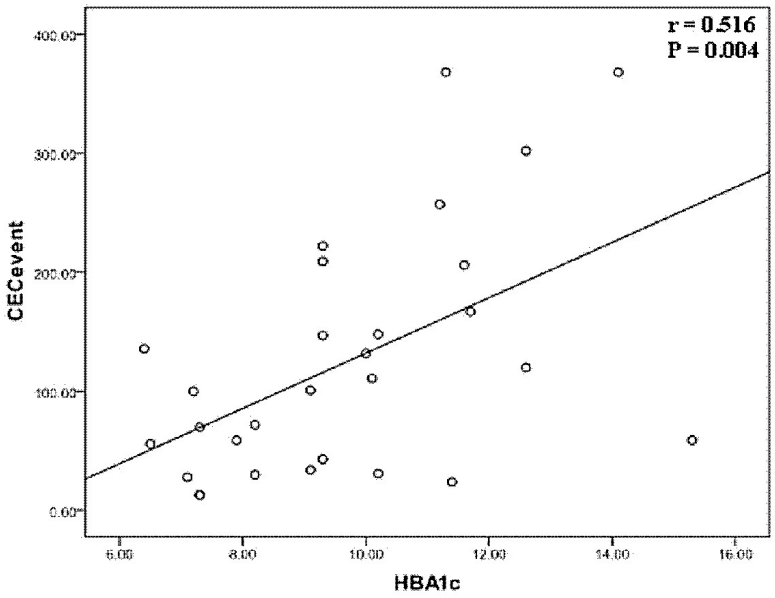

Fig. 3 Correlation coefficient showing the positive relation between circulating endothelial cells (CECs) and HbA1c

hsCRP were significantly higher in the children with T1DM than in the control children. These findings are consistent with those of previous studies [3, 17, 52], which demonstrated increased systemic inflammation in children and adolescents with T1DM. Taken together, our data extend previous findings in T1DM patients ranging from adolescents to preadolescents and suggest that conditions for the early clinical manifestations of atherosclerosis are evident among very young children with T1DM.

This study found that the patients with T1DM had a significantly greater number of CECs than the control subjects. Furthermore, the CEC numbers were positively correlated with HbA1c levels. These results are in accordance with those of a previous study [1]. Prolonged hyperglycemia impairs endothelial cell function, resulting in endothelial dysfunction. The incidence of coronary artery disease, hypertension, and hyperlipidemia is higher in type 2 diabetic patients, and all these disorders have the potential to cause endothelial dysfunction and increased CEC numbers [44].

Furthermore, patients using angiotensin-converting enzyme inhibitors and statins, medications known to alter endothelial function were included in the study. Patients with type 2 diabetes mellitus (DM) also have insulin resistance, which contributes to inflammation, and this inflammation-mediated endothelial dysfunction is substantially less likely to be correlated with HbA1c.

The current study demonstrated that children with T1DM had a significantly impaired brachial artery FMD response and an increased carotid IMT compared with the control group. The diabetic children showed a significant negative correlation between impaired FMD response and increased carotid IMT. Our results were similar to those observed in previous studies [20, 26]. Both increased IMT and impaired FMD have been detected in young children with risk factors for atherosclerosis such as familiar hypercholesterolemia and diabetes [22, 51].

The aforementioned result is consistent with the hypothesis that endothelial dysfunction is a risk factor for the development of atherosclerosis and important in the pathogenesis of premature macrovascular disease in individuals with diabetes. In this study, a significant correlation between plasma level of vitamin C and carotid IMT was detected in the diabetic children, consistent with the findings of a previous study [40] that demonstrated a higher carotid IMT among patients in the lowest tertile of vitamin C than among those in the highest tertile.

Using conventional echocardiography in this study, we found no significant differences in LV systolic function, as reflected by EF and FS, between the children with T1DM and the control subjects. However, the LV diastolic function, as reflected by *E* velocity, *E/A* ratio, IVRT, and DcT, was significantly impaired among the diabetic children. This accords with previous studies [21, 31, 58], which demonstrated that LV diastolic dysfunction with preserved systolic function is common among diabetic children. The LV diastolic dysfunction in patients with DM may be caused by increased LV diastolic stiffness, deposition of advanced glycation end products, and cardiac fibrosis, all as a consequence of DM [56]. This diastolic abnormality appears related to interstitial collagen deposition and LV hypertrophy, which appear in the absence of hypertension [13].

Previous studies have reported a change in early diastolic ventricular function in young adults who have T1DM with normal EF [9, 10]. The relationship between LV diastolic dysfunction and the duration of DM demonstrated that a duration of DM longer than 4 years was correlated with significant LV diastolic dysfunction [15]. These findings disagree with our results, in which LV diastolic dysfunction was detected with systolic function preserved during illness lasting less than 4 years.

The basic manifestations of dystrophic changes in the heart of children with T1DM are disturbances of repolarization and depolarization processes, including prolongation of heart rate QTc intervals. This study confirmed that T1DM patients have significant QTc prolongation (>0.44 s) compared with control subjects. Previously published data demonstrated that prolongation of the QTc interval is a significant predictor of adverse cardiovascular prognosis in patients with T1DM and may be detected in young patients with T1DM [6, 16]. In the current study, the QTc interval was greater in the diabetic children with a low plasma level of vitamin C, and our results are in agreement with another report [40] describing a negative correlation of the QTc interval with the plasma level of vitamin C in children with T1DM.

In experimental studies with rabbit models of T1DM, the QTc interval and the action potential duration were considerably prolonged. The rapid delayed rectifier K+ current (IKr) was markedly reduced in insulin-dependent DM hearts (IDDM), and hyperglycemia depressed the function of the human ether-related gene (hERG), which conducts IKr. The impairment was primarily ascribed to the enhanced oxidative damage to the myocardium, as indicated by the increased intracellular level of reactive oxygen species and the simultaneously decreased endogenous antioxidant reserve and by the increased lipid peroxidation and protein oxidation. Moreover, IDDM or hyperglycemia resulted in downregulation of the hERG protein level [60]. Findings show that hERG potassium channels are essential for normal electrical activity in the heart, and different factors influencing hERG could lead to prolongation of the QTc interval [47]. Vitamin C, because of its excellent antioxidant properties, might affect hERG potassium channels, which would explain the prolonged QTc interval with the low plasma level of vitamin C.

Although the QTc interval was prolonged in the ECG, the LV systolic function was normal, as reflected by EF and FS. However, the QTc interval was significantly correlated with the LV diastolic function. A linear association between prolonged QTc duration and reduced *E* velocity, decreased *E/A* ratio, and prolongation of IVRT and DcT, all markers of abnormal myocardial relaxation and diastolic dysfunction, was found.

In a previous study of patients with hypertension and LV hypertrophy, a modest correlation between the traditional Doppler parameters of diastolic dysfunction (*E/A* ratio and IVRT) and QTc ($r = -0.33$ and 0.35, respectively) was found, supporting the association between diastolic dysfunction and QTc [37]. Electrocardiographic findings that can predict diastolic dysfunction on echocardiography are important given the diagnostic and prognostic implications of diastolic dysfunction [9]. We hypothesized that the QT interval would be most closely associated with LV diastolic dysfunction given the temporal alignment of electrical repolarization and mechanical relaxation in diastole.

To our knowledge, the relation between myocardial repolarization as reflected by the QTc interval and vascular functions as reflected by FMD and carotid IMT, indices of early atherosclerosis, has not been studied in children with T1DM to date. The current study demonstrated a significant linear association between prolonged QTc interval with reduced FMD and increased carotid IMT. A previous study of obese children also found that QTc was significantly affected by carotid IMT [2]. In a study involving clinically healthy adults undergoing cardiovascular risk screening, the results demonstrated that QT and QTc prolongation are associated in part with IMT of the carotid arteries, which is an established risk marker of subclinical atherosclerosis in adults [55].

Conclusion

Alteration in myocardial function and vascular endothelial dysfunction induced by DM may begin early with the association of early atherosclerotic changes. These changes are accelerated when glycemic control is poor. We recommend early and close observation of children with diabetes for any alterations in cardiac function and for vascular endothelial dysfunction. Also, vitamin C supplementation may reduce the risk of complications.

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