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Assessment of Endothelial Dysfunction, Coronary and Carotid Atherosclerosis In Type I Diabetics.

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ABSTRACT

Type I diabetes increases the risk of coronary artery disease (CAD) , and minimal information is available in relation to early history of this process, we aimed to assess the early atherosclerotic burden in diabetic adolescents and its relationships to cardiovascular risk factors. 62 type 1 diabetic patients and 30 healthy volunteers of the same age and sex were included in the study . Blood samples were taken for assessment of glycosylated hemoglobin, and lipid profile.Urine samples were taken for analysis of albumin / creatinine ratio. multislice CT (MSCT) coronary calcium score, carotid intima- media thickness (cIMT) and flow mediated dilatation (FMD) via ultrasound were done. Patients mean age was 16.3 ± 1.5yrs and mean duration of diabetes was 9.4 ± 2.9 yrs. cIMT was significantly higher, while FMD and FMD/ nitrate mediated dilatation (NMD) ratio were significantly lower in diabetics. Rt. cIMT had a significant negative correlation with FMD and FMD/ NMD. cIMT had a significant correlation with waist circumference, waist/height ratio, albumin/ creatinine ratio, total cholesterol and triglyceride . Five patients had positive coronary calcium score (8.1%). Coronary artery calcification (CAC) had significant negative correlation to FMD (p=0.002) and FMD/NMD (p=0.0001). FMD decreased and cIMT increased in diabetic patients, and it is associated with increased incidence of coronary artery disease. These findings indicate endothelial dysfunction and early atherosclerosis. Frequent follow up of type I diabetic patients early for early detection of cardiovascular complications and treatment is recommended.

Keywords: atherosclerosis , coronary calcium score , cIMT , FMD

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INTRODUCTION

Atherosclerosis is an indolent long-term, with significant plaque formation mostly preventable disease in the early years. In the second through sixth decades of life, prevalence of the disease with significant plaque formation is 17%, 37%, 60%, 71%, and 85%.[1]

Many longitudinal epidemiologic studies, such as the Framingham Heart Study, have evaluated atherosclerotic risk factors and defined advancing age, male sex, diabetes, hypertension, dyslipidemias, cigarette smoking, and family history as predictors for cardio-vascular disease. Coronary calcium is absent in the normal vessel wall and it occurs exclusively in atherosclerotic coronary arteries.[2]

Juvenile diabetes is a major risk factor resulting in two to four-fold increased risk of developing atherosclerotic diseases.[3] Coronary artery disease occurs two or more decades earlier in diabetics and postmortem studies revealed that atherosclerotic lesions in young adults are associated with the prediabetic state.[4] Senior et al[5] studied asymptomatic type 1 diabetic patients of older age group (24–64years old) and reported that 43% of these asymptomatic subjects had coronary artery stenosis >50%. So, diabetic children are at high risk of accelerated development of atherosclerosis and its complications.[5]

Atherosclerosis is the only disease associated with coronary calcification and is intimately associated with plaques.[6,7] The most important application of the multislice CT CAC (coronary artery calcium) examination is the high negative predictive value of a zero CAC score that indicates no calcium is present and consequently indicates that there is little likelihood of significant arterial stenosis (negative predictive value 95% to 99%). A negative score is consistent with a low risk for hard coronary event (0.1% per year) or any event in the next 2 to 5 years.[7] So in a diabetic population, patients with a high risk for future myocardial infarction (MI) and coronary disease (CD) could be identified by the determination of coronary calcifications independent of concomitant cardiovascular risk factors. Thereby the Agatston score showed higher diagnostic accuracy in predicting MI compared to Framingham risk score. [8]

Multislice computed tomography offers the possibility to detect and quantify the amount of coronary calcium. In histopathologic studies, coronary calcification have shown to be a sensitive marker of early stages of coronary atherosclerosis.[9,10] Furthermore, the amount of coronary calcifications correlates to the extent of coronary atherosclerosis and coronary stenosis.[11,12] The earliest functional atherosclerotic changes in the arterial wall is the endothelial dysfunction due to impaired endothelial release of nitric oxide detected by measuring flow mediated dilatation (FMD) of the brachial artery that can be assessed by measuring arterial diameter responses to increased flow.[13] Measurements of carotid intima-media thickness (cIMT) via B-mode ultrasound is a safe, simple, and inexpensive method for evaluating cardiovascular (CV) risk by measuring the combined thickness of the intimal and medial layers of the arterial wall. The using of cIMT testing can also detect marked thickening of the arterial wall, possibly indicating plaques or atheromas that are associated with accelerated atherosclerotic disease and increased risk for coronary artery disease, myocardial infarction, and stroke.[14] Both increased IMT and impaired FMD have been detected in young children with risk factors for atherosclerosis, such as diabetes.[15,16]

Cohen [17] reported endothelial dysfunction is the earliest event in the atherosclerotic process and Järvisalo et al.[18] found impaired FMD response is a common manifestation in children with type 1 diabetes and associated with high carotid artery IMT, suggesting that endothelial dysfunction in with type 1 diabetics may predispose them to the development of early atherosclerosis.

The objective of this study is to assess early atherosclerotic burden in diabetic adolescents early & its relationships to cardiovascular risk factors.

Patients and Methods:

Patients:

The study included 62 adolescent patients with type 1 diabetes mellitus (DM) among those attending to the endocrine clinic, National Research Centre. The control group consisted of 30 age and sex matched healthy normal volunteers. Control group was the healthy friends or relatives of our patients.

Inclusion criteria: children with type 1 DM, duration of disease > 5 years, patients age > 14 and < 19 yrs old. We selected this young age group with short duration of diabetes firstly, to explore whether early atherosclerotic changes starts at this early age shortly after onset of diabetes or needs longer exposure to the diabetic milieu and secondly because in younger age group (< 14 yrs old) atherosclerotic lesions are expected to be in the form of microscopic intimal fatty streaks that is too minute to be resolved by ultrasonography.

Exclusion criteria were: patients during acute diabetic complications e.g. diabetic ketoacidosis (DKA) or hypoglycemia, patients suffering from cardiac diseases e.g. congenital, rheumatic heart, left ventricular dysfunction, patients on metformin or multivitamins and smokers.

Study design and protocol:

It is a cross-sectional observational study done after obtaining approval from the ethical committee of the National Research Centre, Cairo, Egypt. Registration number is 11052. Written informed consent was obtained from all patients or their parents and controls after full discussion about the aim of the study. This study is a part of a project done in the National Research Centre for evaluation of cardiac, vascular and endothelial function in adolescent type 1 diabetic patients.

All the studied patients were subjected to:

History taking including: Age of patients, sex, age of onset of diabetes, duration of diabetes, type and dose of insulin therapy, family history of diabetes.

We asked about presence of any symptoms of cardiac, renal, neurological affection or presence of any type of autonomic dysfunction. We also asked about history of taking drugs other than insulin.

Clinical examination:

I. Patients and controls were subjected to general, cardiac, chest and neurological examination.
II. Blood pressure was measured three times for patients and controls after 5-minute rest in the sitting position on both upper limbs with the use of automatic manometer (Omron M4 Plus, Omron Health care Europe, Hoof drop, and Holland). The mean value of the second and the third measurement was calculated. The measurements taken on the dominant limb were analyzed.
III. Anthropometric measurements in the form of weight, height, waist circumference (WC), and hip circumference (HC) were taken for each participant. The weight and height of the participants were measured up to 0.01 kg and 0.1 cm using a Seca Scale Standing Balance and a Holtain Portable Anthropometer (Holtain, Ltd, Crymmych, Wales, U.K.). Body mass index (BMI) was calculated as weight (in kilograms) divided by height (in meters) squared. Waist circumference was measured at the level of the umbilicus with the participant standing and breathing normally; hip circumference was measured at the level of the iliac crest, using non stretchable plastic tape to the nearest 0.1 cm. The waist / hip ratio and waist / height ratio (cm/ cm) were calculated. Each measurement was taken as the mean of three consecutive measurements, using standardized equipment.[19,20] The landmarks, instruments used, and techniques followed were those recommended by the international biological program. [19,20]

Laboratory investigation:

Simultaneously all patients and controls underwent the following tests:

I. For cholesterol measurements, venous blood was sampled after 12 hr fast. Serum total cholesterol was determined by a commercial kit (Boehringer-Mannheim, Germany). High-density lipoprotein (HDL) cholesterol was separated from the serum by precipitation of the other lipoproteins with a heparin/manganese procedure.[21] Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald equation. The concentration of triglycerides (Tg) was measured in a TechnoConAutoAnalyzer II (TechnoCon Instruments, Tarrytown, New York).

II. Glycosylated hemoglobin (HbA1) was done every 3 months and the mean value was calculated per year. It was determined spectrophotometrically using commercially kit supplied by Stanbio, USA according to the method described by Trivelli et al. [22]
III. Screening for microalbuminuria was assessed in fresh morning urine samples by measuring albumin/creatinine ratio by enzyme linked immunosorbent assay (ELISA) kit provided by (OrgentecDiagnostika,Gmbh, Mainz, Germany).[23]

Carotid intima-media thickness (IMT) assessment:

A single experienced vascular sonographer, who was blind to the clinical and laboratory data of the study subjects, performed all imaging studies. The images were obtained using (General Electric medical ultrasonographic machine model: Vivid 7 Pro, GE Vingmed ultrasound AS-Nl90, Horton-Norway equipped with 7.5–10 MHz linear-array transducer). Imaging of the carotid arteries is performed in the cardiovascular ultrasound laboratory with the subject resting in the supine position with his/her neck extended, and the head turned 45° toward the contralateral side. Care was taken to have the vessel as perpendicular as possible to the plane of ultrasound beam to ensure optimal imaging of the vessel wall in its longitudinal axis with the least possible pressure in order not to compress the overlying jugular vein and to allow expansion of the carotid artery in all directions. A longitudinal section of the common carotid artery 1 cm proximal to the carotid bulb was imaged to achieve consistent site of measurement, and a resolution box function was used to magnify this part of the artery. Three maximal IMT measurements of the far wall of the artery at 3-mm intervals were obtained starting at 1 cm proximal to the bulb and moving proximally. The reported IMT for each side is the average of these 3 measurements and the reported IMT for each subject is the average of the 6 measurements (3 measurements from the right and 3 from the left common carotid artery). Generally, images are recorded in the plane where the maximal cIMT can be visualized. Magnification of the vessel wall allows easy identification of the intimal-medial complex, defined by the border between the echolucent vessel lumen and the echogenic intima and the border between the echolucent media and echogenic adventitia. Image frames are selected on the basis of areas where the intima-media complex is best visualized and appears the thickest, irrespective of the cardiac cycle, with manual assessment by the sonographer using electronic calipers online.

Flow mediated dilatation (FMD)

All imaging studies were performed by the same vascular sonographer & the same ultrasonographic machine using (General Electric medical ultrasonographic machine model: Vivid 7 Pro, GE Vingmed ultrasound AS-Nl90, Horton-Norway equipped with 7.5–10 MHz linear-array transducer) after the published protocols.[24-26] With the subject lying in the supine position, ECG electrodes were placed on the chest; the machine automatically measured and recorded results of the electrocardiogram. All measurements were made at end diastole to avoid possible errors resulting from variable arterial compliance. A sphygmomanometer cuff was placed on the proximal right arm. The right brachial artery images were obtained 3cm proximal to the elbow crease using B-mode imaging in the longitudinal plane of the artery. A baseline image was acquired using a resolution box function to magnify this part of the artery. Blood flow was estimated by the pulsed Doppler velocity signal obtained from a mid-artery sample volume. The cuff was inflated to 100 mm Hg above the systolic pressure to occlude arterial flow for 5 min. The cuff was then deflated, and the longitudinal image of the brachial artery was recorded immediately & continuously for 60 seconds after cuff deflation for greatest response guided by the hyperemic flow detected by pulsed Doppler. Flow-mediated dilation (FMD) was assessed by measurement of the greatest brachial artery diameter that was detected at 60 seconds after release of the cuff in most cases. The subject then had a rest for 30 min, after which a sublingual dose of nitroglycerin tablet (Dinitra, isosorbidedinitrate 5mg manufactured by Egyptian int. pharmaceutical industries co., tenth of Ramadan city, A.R.E., under license of RHONE POULENC, PARIS FRANCE) was then administered, and the brachial artery response (endothelium-independent dilation) was assessed by imaging the artery continuously for 3 min after the nitroglycerin dose for greatest response.

Measurements of the brachial artery luminal diameter were performed on-line at end-diastole, coinciding with the onset of the R-wave on the ECG. For each phase (baseline, endothelium-dependent dilation, and endothelium-independent dilation), three brachial artery diameter measurements were obtained manually online with electronic calipers and averaged from the longitudinal image by identifying the lumen-intima interface. The largest reading for FMD post-ischemia {100 × [diameter (1 min)–diameter (basal)]/diameter (basal)} was used to represent spontaneous endothelial function. In addition, nitroglycerine-

mediated dilatation (NTG) {100 × [the largest reading of diameter (after sublingual isosorbidedinitrate–diameter (basal)]/diameter (basal)} was assessed. The diameter percent change caused by endothelium-dependent flow-mediated dilatation (%FMD) and non-endothelium dependent dilatation (%NMD) were expressed as the percent change relative to that at the initial resting scan. Significant endothelial dysfunction was defined as FMD < 10% and NMD > 10%.[27] In order to increase the sensitivity and specificity of the technique for endothelial dysfunction, FMD over NMD of the brachial artery < 0.70 defined endothelial dysfunction.[28]

Assessment of coronary calcium scoring by multisclice CT:

CT examination was performed in one center using the CT scanner; iCT 256 (Philips Medical Systems; Eindhoven, Netherland).

The patient is positioned supine on the CT table. ECG leads are fixed at the four corners of the pericordium. All reconstructions are performed using the retrospective ECG gating. For this technique; an ECG must be recorded simultaneously throughout the duration of the scanning.

First; a localization scan (scanogram) is performed that yields an antero-posterior and lateral views of the chest. It is used to position the imaging volume of the coronary arteries that extends from the level of the carina down to about 1 cm below diaphragm. The center of the field of view is 2 cm to the left of the dorsal spine on the AP scout and at the level of the hilum on the lateral scout.

A non-contrast CT examination of the heart was performed for all patients in order to detect and quantify coronary calcifications through the volume extended from below the carina to the apex of the heart. Acquisition parameters were ECG gated at 75% of the RR interval, 270 ms gantry rotation, 256 x 0.625 mm collimation, 80 mA, and 120 kV. To minimize the total effective patient radiation dose, this stage of the scanning was conducted with a relatively low tube current.

The radiation dose of the CT coronary calcium score, according to this technique, is about 1 mSv, in average. A radiologist read all computed tomography using an interactive scoring system similar to that used by Yaghoubi et al.[29]

Statistical Analysis:

Statistical analysis was conducted using Statistical Package for Social Science (SPSS) program version 15.0 (Chicago, Illinois, USA). t –test or Mann Whitney-U (for non symmetrically distributed data) for independent variables was done. Pearson's or Spearman correlation were also used.

RESULTS

The study included 62 patients with type1 diabetes (31 males and 31 females) and 30 healthy volunteer (15 males and 15 females). The mean age of patients were 16.3 ± 1.5yrs and mean duration of diabetes were 9.4 ± 2.9 yrs. HbA1, albumin/creatinine ratio, cholesterol, Tg, and LDL were significantly higher in diabetic patients (data not shown). cIMT was significantly higher (fig. 1), while FMD and FMD/ nitrate mediated dilatation (NMD) ratio were significantly lower in diabetics (fig. 2 and 3). On the other hand, NMD showed no significant difference. Right cIMT had a significant negative correlation with FMD and FMD/ NMD. cIMT had a significant correlation with waist circumference, waist/height ratio, albumin/ creatinine ratio, total cholesterol and triglyceride (data not shown). HbA1 relatively increased in our diabetic patients with increased cIMT but the relationship between HbA1 and cIMT in diabetic children didn't reach statistical significance. No significant difference of demographic and anthropometric data was found in diabetic patients in relation to CAC score. Five patients had positive coronary calcium score (8.1%) (fig.4). CAC had significant negative correlation to FMD (p=0.002) & FMD/NMD (p=0.0001). Table 1 showed individual data of diabetic patients with positive coronary calcium score. Table 2 showed comparison between FMD and carotid intimal medial thickness in relation to coronary calcium scoring.

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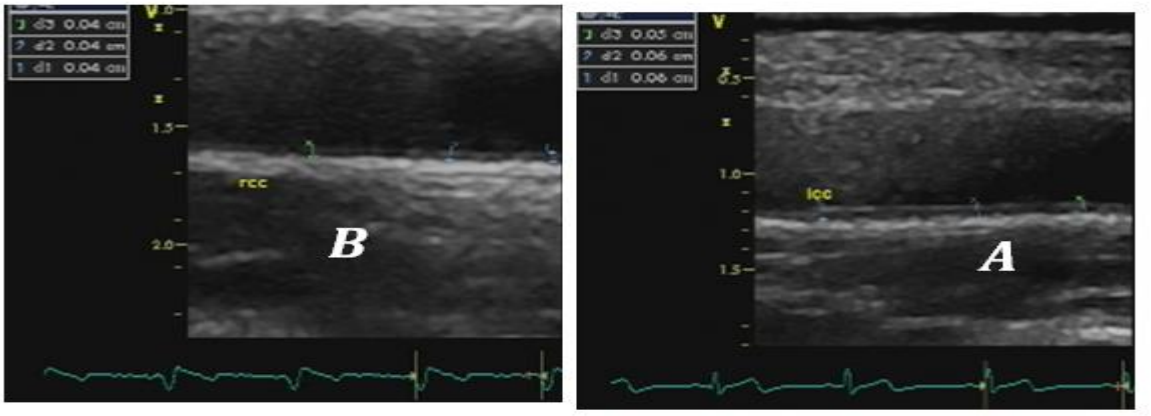

Fig 1 : Carotid intimal medial thickness in control (A) and diabetic patients (B)

A: showing normal cIMT of one of the controls, measured during diastole. cIMT= 0.4mm.

B: showing abnormal cIMT of one of the patients, measured during diastole. cIMT= 0.6mm.

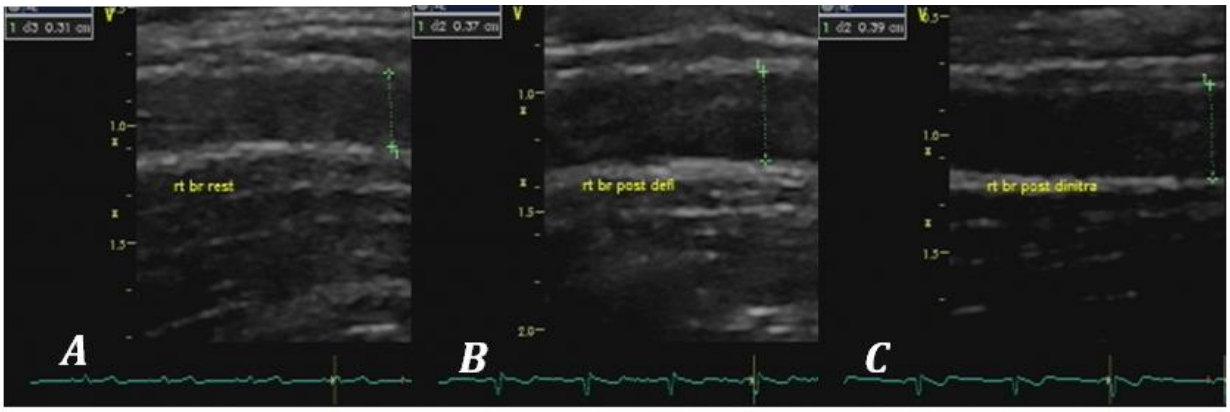

Fig 2: Flow mediated dilation (FMD) and nitrate mediated dilatation (NMD) of one of the controls. Diameter of brachial artery was measured during diastole.

A, diameter of brachial artery at rest measures 3.1mm.

B, diameter of brachial artery after cuff deflation measures 3.7mm.

C, diameter of brachial artery after sublingual dinitrameasures 3.9mm.

FMD measures 19.4% and NMD measures 25.8%.

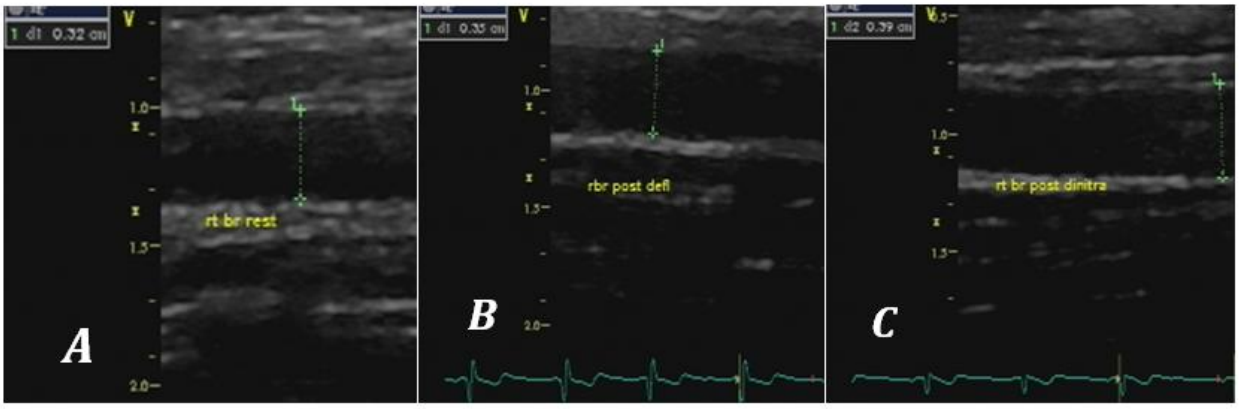

Fig 3: Abnormal Flow mediated dilation (FMD) and normal nitrate mediated dilatation (NMD) of one of the patients. Diameter of brachial artery was measured during diastole.

A, diameter of brachial artery at rest measures 3.2mm.

B, diameter of brachial artery after cuff deflation measures 3.5mm.

C, diameter of brachial artery after sublingual dinitrameasures 3.9mm.

FMD measures 9.4% and NMD measures 21.9%.

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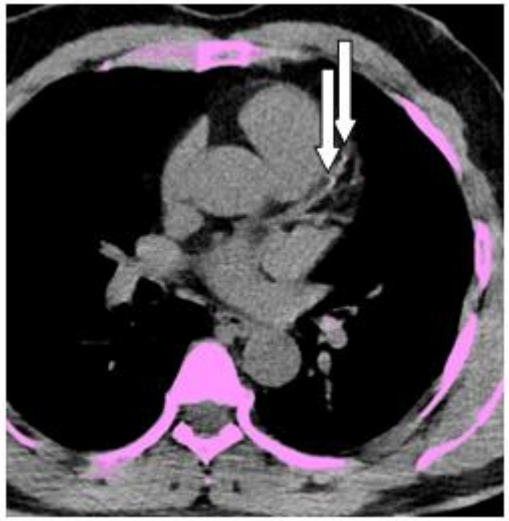

Fig (4): Mutislice CT (MSCT) coronary angiography showing two small calcified plaques at the mid left anterior descending (LAD) artery in one of the diabetic patients. He has no other coronary calcifications. The total coronary calcium score for this patient was 5.

Table 1: Individual data of diabetic patients with positive coronary calcium score

No	Sex	Age (yrs)	Duration of DM (yrs)	Onset of DM (yrs)	Systolic BP (mmHg)	Diastolic BP (mmHg)	RT cIMT	LT cIMT	Brachial cIMT	Chole--sterol (mg/dl)	Triglyce-rides (mg/dl)	HDL (mg)	LDL (mg)	HBA-1 (%)	Alb / creatinine ratio (µg/g creatinine)	Capoten	Lipitor
1	M	17.5	6	11.5	140	80	0.6	0.5	6.1	170	120	26	140	8.4	40	yes	No
2	F	19	9	10	140	110	0.4	0.4	5.6	130	140	44	58	13.2	40	yes	yes
3	M	17	12	5	110	80	0.5	0.5	5.3	163	143	73	81	9.7	15.6	No	No
4	M	14.9	13	1.9	110	70	0.6	0.6	2.7	160	90	47	95	5.9	13	No	No
5	M	16.5	6.5	10	120	80	0.5	0.5	3.5	172	60	45	115	7.2	9.4	No	No

M: males, F: females, BP : blood pressure, cIMT : carotid intima media thickness, HbA1 : glycosylated hemoglobin, Alb: Albumin.
LDL > 70 mg is abnormal, HbA1 > 7 % is a poor control, albumin / creatinine ratio > 30 is microalbuminuria

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Table 2: Comparison between flow mediated dilatation and carotid intimal medial thickness in relation to coronary calcium scoring

Variables	Negative CCS N = 57		Positive CCS N =5		P-value
	Mean	SD	Mean	SD	
FMD	10.71	7.28	5.66	2.11	0.002
FMD/ NMD	0.88	0.70	0.33	0.08	0.0001
RtcIMT	0.49	0.08	0.51	0.09	0.60
Lt cIMT	0.49	0.08	0.49	0.08	0.90
Both cIMT	0.49	0.08	0.50	0.08	0.70

FMD: flow mediated dilatation, NMD: Nitrate mediated dilataion, cIMT:carotid intimal medial thickness.

DISCUSSION

In our study, HbA1, albumin/creatinine ratio, cholesterol, Tg, and LDL were significantly higher in diabetic patients. Also reduced brachial artery FMD response and increased cIMT were common vascular manifestations in type 1 diabetic children. The attenuated FMD response in diabetic children in the current study is in agreement with the results of Wiltshire et al.,[30] and Donaghue et al.,[31] who studied flow-mediated dilation in diabetic children and demonstrated attenuated endothelial function in diabetic children compared with controls.

The present study showed that diabetic children have significantly increased cIMT compared with normal control. These findings are in agreement with the findings of postmortem studies that have indicated a relation between early atherosclerotic lesions and diabetic state.[32] Also Järvisalo et al.[18] have demonstrated that type I diabetes predisposes to increased subclinical atherosclerosis at a very early age and reported that type 1 diabetes is an independent risk factor for increased cIMT in children.[33] Many previous studies demonstrated that cIMT is increased in adults with type 1 diabetes.[34-38]

In current study, cIMT was significantly correlated with cholesterol and triglyceride levels but no significant relationship could be elicited between cIMT and serum LDL cholesterol concentration. Children have shown a significant relationship between serum cholesterol concentration and early atherosclerotic lesions[39,40] and previous studies in children with familial hypercholesterolemia.[41-43] Also, these studies have not been able to show a significant relationship between cIMT and serum LDL cholesterol concentration within the normocholesterolemic range. Also, Järvisalo et al[18], reported high cIMT in diabetic children and found that total cholesterol and LDL cholesterol concentrations were similar between the diabetic and control groups, but the children with diabetes had increased oxidized LDL. They reported that oxidized LDL was correlated significantly with mean IMT.[33]

Fatty streaks, are commonly found in the arteries of adolescents by histology, whereas the development of raised lesions mainly occurs after the age of 20 years.[44] According to these postmortem findings and according to the significant correlations between lipid risk factors and IMT seen in the present study, it may be suggested that the diffusely increased cIMT in children with diabetes reflects intimal changes related to early atherosclerosis.

In our study, HbA1 levels are increased in diabetic children and relatively increased in our diabetic patients with increased cIMT but the relationship between HbA1 and cIMT in diabetic children didn't reach statistical significance, in the contradictory to previous studies that have emphasized the role of hyperglycemia in explaining the increased cardiovascular morbidity and mortality in diabetes.[45,46] Chronic hyperglycemia induce atherogenesis by increasing oxidative stress, resulting in increased LDL oxidation and decreased nitric oxide bioavailability, including endothelial dysfunction.[47-51] Alternatively, hyperglycemia may exert its effects by promoting glycosylation of LDL, which may increase its atherogenicity.[52,53]

In our study, the increased cIMT in diabetics was positively correlated to waist circumference and waist/height ratio but not correlated to BMI. Rathsman et al. [54] have demonstrated similar results as regards to increased cIMT in diabetic adolescents with positive correlation between cIMT and waist circumference and lack of correlation between cIMT and BMI.[54]

In the current study, cIMT, which is an early sign of atherosclerosis and thereby, sign of macrovascular diseases, was positively correlated to the albumin/creatinine ratio, which is an early sign of diabetic nephropathy and thereby, sign of microvascular diseases. Our results are in agreement with the findings of Gül et al[55] who demonstrated significantly higher cIMT in diabetics compared to control group and significant positive correlation between cIMT and microvascular complications (nephropathy and/or retinopathy).[55] These findings suggest that diabetic microangiopathy and macroangiopathy are related to each other in type 1 diabetic patients.

In the current study, positive coronary calcium score (8.1%) was found in 5 of our patients. A significant negative correlation was found between CAC , FMD (p=0.002) and FMD/NMD (p=0.0001). To date, reports of coronary artery calcifications in pediatric patients are limited. Our results are in agreement with Salem et al[56] that studied coronary artery calcification (CAC) among adolescents with type-1 diabetes with similar age range to our group and half of their patients were smokers. They reported positive CAC in 12 patients (20%) with diabetes, CAC was found in 9.1% of non-smokers with diabetes.[56] Also Starkman et al[57] studied 101 subjects aged 17–28 years and duration of type 1 diabetes > 5 years and reported that eleven subjects (10.9%) had CAC.[57] Thilo et al[58] studied coronary calcium in asymptomatic long-term type 1 diabetic patients in older age group (age 48 +/- 9 y) and longer duration of diabetes (26 +/- 9 y) and reported that coronary calcifications were detectable in 22 (31 %) type 1 diabetic patients that was significantly correlated to microangiopathy and duration of diabetes. On the other hand, age of patients, body mass index, and HbA1c were not significantly different between diabetic patients with and without coronary calcification.[58] In our study, patients with positive CAC were mostly males (80%). Our findings are in agreement with Janowitz et al[59] who reported difference in women, with lower scores in younger patients, but this is eliminated in the 65 to 70 years of age group.[59] There was only one female patient with positive CAC in our study and this patient was hypertensive and hyperlipaemic, on capoten and lipitor medications and was older (19 years old).

In the current study, all patients with positive CAC had endothelial dysfunction, 80% of patients had increased cIMT, 80% had increased LDL, 80% had increased HBA1 (poor glycemic control), 40% were hypertensive and 40% had nephropathy with albumin/creatinine ratio of 40 microgram/gram creatinine. Salem et al[56] reported similar results with higher mean glycosylated hemoglobin, percentiles of blood pressure, albumin/ creatinine ratio and serum lipids were significantly higher in patients with positive CAC score. In our study, a significant negative correlation was found between CAC , FMD (p=0.002) and FMD/NMD (p=0.0001), being the earliest functional atherosclerotic changes in the arterial wall and well correlated to cardiovascular risk factors as we have discussed earlier. Increased cIMT, which is the earliest structural atherosclerotic changes in the arterial wall, is noted in 80% of patients with positive CAC but didn't reach statistical significance due to the small number of patient with positive CAC. If the number of patients increases and we select patients with longer duration of disease, we can find more patients with positive CAC score and a significant difference could be found. ‘

No significant difference of CAC score in relation to demographic and anthropometric data was found in diabetic patients. Our present findings may have implications in studying the origins of vascular disease in type1 diabetes, as well as in the management of pediatric patients with diabetes. Our results emphasize the importance of early detection and control of vascular risk factors in diabetic children to reduce cardiovascular morbidity through close monitoring and follow up of early vascular changes using these non invasive techniques; FMD, cIMT and CAC score. Because diabetics with endothelial dysfunction appear to be at particular risk for developing early structural atherosclerotic changes, the ultrasound assessment of arterial FMD responses might provide a valuable tool for risk stratification of pediatric patients with type1 diabetes.
We conclude that the prevalence of early coronary artery disease as evidenced by CAC in adolescents with type1 diabetes is significant. FMD independently predicts the presence of CAC score. cIMT in patients with type 1 diabetes had a more advanced degree of atherosclerotic changes than healthy controls. FMD in brachial artery is useful in assessing impaired endothelial function in people suffering from the risks factors of atherosclerosis. Ultrasonographic methods of evaluating atherosclerotic changes in arterial vessels should be more often used in practice as relatively easy, non invasive and inexpensive. cIMT, and FMD are affected in diabetics. Affected cIMT is associated with endothelial dysfunction. Several interventions to improve endothelial dysfunction, including antioxidant, vitamins and statins, have been tested in diabetic adults and in children with familial hypercholesterolemia.[60-62] We recommend such interventions to be studied in the future in diabetic children to examine whether improvement of arterial endothelial function in these children would translate into slowing down or regression of the atherosclerotic process.

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