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Original Research

Levels of endothelial progenitor cells in children and adolescents with type 1 diabetes; an early marker for detection of cardiovascular complications

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Abstract

Background: Type 1 diabetes mellitus (T1D) is a chronic metabolic disorder characterized by chronic hyperglycemia. T1D in children and adolescents is considered a high-risk factor for the development of cardiovascular disease afterward in adulthood. Endothelial progenitor cells (EPCs) number and function were found to influence endothelial function and vessels repair. Carotid intima medial thickness (CIMT) measurement has been a method to detect subclinical cardiovascular changes in children and adolescents with T1D, along with lipid profile and markers of glycemic control. **Methods:** Sixty children and adolescents with T1D with disease duration exceeding 5 years were recruited from Diabetes clinic Ain Shams University Hospitals. They were compared to 60 age and sex-matched healthy controls attending the outpatients' clinics. All subjects were subjected to history taking, examination and blood sample withdrawal for – EPCs, lipid profile; total cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, as well as mean random blood sugar (RBS) and mean HBA_{1c}. Also, early morning samples of urine were collected for microalbumin, measurement of carotid intema media thickness was done and nerve conduction velocity study was performed on all patients as well as fundus examination. **Results:** EPCs number and percentage were significantly lower in the patients' group (p=0.001). The mean CIMT in the patients' group was significantly higher in the patients' group compared to the control group (p=0.003). Moreover, the patients' group had significantly higher levels of total serum cholesterol (p=0.001) and LDL-cholesterol (0.015), and lower levels of HDL-cholesterol (p=0.003) than the control group. There was a statistically significant negative correlation between EPCs percentage and mean RBS (p=0.022), mean HbA_{1c}% (p=0.013), the presence of diabetic retinopathy (p=0.001) but not with diabetic nephropathy nor diabetic neuropathy, total serum cholesterol (0.003), LDL cholesterol (p=0.015) and mean CIMT (p=0.021). **Conclusion:** EPCs percentage is a good predictor for increased CIMT in children with T1D. Yet, further longitudinal studies are needed to confirm this relationship. Children and adolescents with T1D are at increased risk for atherosclerosis and macrovascular complications supported by the increased levels of harmful lipids (total serum cholesterol, triglycerides, LDL cholesterol) and increased mean CIMT. This may contribute to new strategies for early detection of diabetes-related complications and intervention to prevent macrovascular complications.

Keywords: diabetes complications, type 1 diabetes, endothelial progenitor cells, carotid intima media thickness, cardiovascular risk.

Introduction

Type 1 diabetes (T1D) is a chronic metabolic disorder resulting from insulin insufficiency. Autoimmune destruction of the pancreatic beta cells is the trigging factor for pancreatic disruption [1]. Hyperglycemia is the main feature in T1D, it is responsible for the characteristic clinical picture of the disease as well as the development of various complications [2]. The mechanisms whereby high glucose levels damage the microvasculature are well-known in literature: the pathway leading to the formation of advanced glycation end-products; activation of the polyol pathway and increased production of glucosamine [3]. Hyperglycemia is also known to induce overexpression of mitochondrial reactive oxygen species. Angiogenic factors that affect blood vessel formation are also important players in the development of microvascular complications [4].

Endothelial progenitor cells (EPCs) are circulating immature cells that contribute to vascular homeostasis and compensatory angiogenesis. EPCs have the ability to differentiate into mature endothelium and take part in neoangiogenesis. These cells co-express surface markers of both hematopoietic stem cells (CD34 and CD133) and endothelial cells (CD146, Vwf, and VEGFR2) [5, 6]. It is worth mentioning that these cells are derived from bone marrow and can be mobilized to the peripheral circulation in response to various stimuli; C-reactive protein has a direct role in the reduction of EPCs number and activity, influencing adhesion through a reduction tumor of mRNA transcription of chemo attractant factors. Similarly, tumor necrosis factor-alpha (TNFα), with its well-known myelosuppressive effect, could be responsible for the reduction of hemopoiesis and EPCs levels observed in the late phases of heart failure [7].

This pool of circulating endothelial cells exerts a pivotal function as an endogenous repair mechanism to keep the healthiness and integrity of the endothelial monolayer by replacing damaged areas of an artery. The maintenance of the endothelial monolayer may prevent thrombotic complications and atherosclerotic lesion development [8]. A severely impaired re-endothelialization capacity of EPCs in patients with T1D might be due, at least in part, to an increased NADPH oxidase-dependent superoxide production and subsequently reduced nitric oxide bioavailability [9].

Data has shown that low levels of EPCs in patients with T1D may have an important causative role in the development and progression of almost all diabetes complications [10]. An association was found between reduced levels of EPCs and impaired micro- and macrovascular function in children and adolescents with T1D [11]. Circulating EPCs are reduced in youth with T1D without overt vascular injury and this is associated with impaired endothelial function and increased carotid intima-media thickness (CIMT) [12].

Subjects and methods

This pilot study was conducted on one hundred and twenty (120) children and adolescents recruited from Children's Hospitals, Pediatric department, Faculty of Medicine, Ain Shams University Hospitals, Cairo, Egypt. Patients' group included sixty (60) children and adolescents with T1D attending the Pediatric and Adolescents Diabetes Clinic, Ain Shams University Hospitals.

Patients diagnosed as T1D in Diabetes Clinic according to ISPAD diagnostic criteria of diabetes 2018. Patients' ages ranged from eight to seventeen years (mean age=12.7 years). This study did not include patients with type 2 diabetes mellitus or other types of diabetes. Also, patients with infections, inflammatory conditions, liver diseases, kidney diseases, and cardiovascular diseases were excluded. The patients' group was subdivided further into 2 groups according to the HbA_{1c} levels; group I with HbA_{1c} less than 7.5 gm% and group II with HbA_{1c} more than 7.5 gm%. The control group included sixty (60) age and sex-matched healthy children and adolescents. They were recruited from Outpatient Clinic at Ain Shams University Hospitals.

Written informed consent was taken from the care-givers of participants with ages below sixteen, and taken from participants for ages equal to or more than sixteen. The procedures applied in this study were approved by The Ethical Committee of Human Experimentation

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of Ain Shams University, and are in accordance with the Helsinki Declaration.

Data collected from all patients in the study included detailed medical history taking with special stress on – demographic data: name, age, sex, family history of diabetes, disease duration, history of DKA, and/or hypoglycemic attacks and presence of diabetic complications.

All subjects included in the study had a thorough full clinical examination laying stress on: Anthropometric measures were taken including weight in kilograms (kg) and height in centimeters (cm). Body mass index (BMI) was calculated as kg/m^2 and plotted on the age – and sex-standard percentiles according to WHO growth charts for weight, height, and BMI Z scores [13]. Neurological examination was done to detect any signs of neuropathy in the form of loss of peripheral deep tendon reflexes (ankle reflex), loss of vibration sense (using 128 Hz tuning fork on medial malleolus), loss of light touch sense or temperature in the upper limbs and lower limbs extremities. Nerve conduction velocity test was performed to assess neuropathy in patients' group in neurology department Ain Shams University using DANTEC counter print Mk II. Nerve conduction velocity was performed on the median (upper limb) and tibial (lower limb) nerves on the non-dominant side with the band-pass filter set at 5 Hz–5 kHz. Compound muscle action potential (CMAP) was recorded from a pair of surface cup electrodes placed over the target muscles. Square pulse supramaximal electrical stimuli with a duration of 0.5 ms were delivered at the wrist and elbow to the median nerve and at the ankle and popliteal fossa to the tibial nerve [37]. A slow nerve conduction measure was defined as being lower than normal range values for median and tibial nerves for age [39]. Finally, fundus examination was done by direct ophthalmoscope to detect retinopathy by an experienced ophthalmology specialist in Ain Shams University hospital, ophthalmology department.

Blood sampling and laboratory analysis

Under complete aseptic conditions, 10 ml of fasting venous blood was obtained by a clean venipuncture, two milliliters was placed in EDTA tube for subsequent assay of HbA_{1c}, while the rest was evacuated in two plain test tubes. The serum was separated by centrifugation (1000× g for 15 minutes). The serum of one tube was immediately assayed for lipid profile, while the serum collected in the other tube was divided into three aliquots and stored at -20°C for subsequent assay of fasting C-peptide, serum lipase, and trypsinogen. Hemolysed samples were discarded. Repeated freezing and thawing were avoided.

HbA_{1c} was assayed by high-performance liquid chromatography (HPLC) technique on the Bio-Rad d-10 hemoglobin testing system (Bio-Rad Laboratories, Inc., 4000 Alfred Nobel Drive, Hercules, California 94547, USA). Serum samples were assayed for lipid profile on the Beckman AU-680 system auto-analyzer (Beckman Coulter, Inc. Diagnostics Division Headquarters 250 South Kraemer Boulevard Brea, California 92821-6232 USA) using reagents supplied by the company. Triglycerides (TG) and total cholesterol were measured on AU680 are based on the enzymatic colorimetric method, high-density lipoprotein cholesterol (HDL-cholesterol) assay was based on precipitation of low-density lipoprotein cholesterol (LDL-cholesterol) and very-low-density lipoprotein cholesterol (VLDL-cholesterol) and then the cholesterol in the HDL cholesterol fraction which remains in the supernatant is assayed by a timed endpoint method. LDL cholesterol calculated according to "Friedwald equation": LDL-cholesterol = Total cholesterol-(HDL-C+TG/5), provided that serum triglycerides (TG) are ≤400 mg/dl, samples with TG>400 mg/dl are diluted according to its concentration.

Albumin excretion rate (AER) – using immune turbidimetric methods. It is used to assess the presence of early nephropathy. Patients initially detected as having AER >30 μg/mg creatinine were asked to perform two further urine collections at intervals of 3–6 months. Persistent microalbuminuria was defined when two of three samples showed an excretion rate of more than 30 μg/mg of creatinine.

EPCs were measured in a heparinized blood sample by flow cytometry. Flow cytometry is based on the measurement of multiple cellular properties; optical and fluorescence characteristics on single-cell basis as cells are moving in a fluid stream and interrupting as a laser beam. This was done using flow cytometry apparatus [Coulter EPICS XL (Beckman Coulter, Florida, USA)] for detection of membrane antigens. The percentage of cells expressing the certain antigen and the mean fluorescence intensity is calculated by the software (system II™ version 3.0) incorporated in the FCM. Results are expressed as the percentage of cells expressing each antigen tested out of the gated cells, (and the intensity of staining whether dim or bright).

In this study EPCs were measured in a heparinized blood sample by flow cytometry to assess coexpression of CD34/KDR and CD133 using the procedures to detect membrane surface antigens; CD133/2 (293C3) antibodies human (1 ml contains monoclonal CD133/2 (293C3) antibodies conjugated to R-phycoerythrin (PE) and CD34 antibody human (1 ml contains monoclonal CD34 antibodies conjugated to fluorescein isothiocyanate (FITC).

Imaging

CIMT was measured using high-resolution ultrasound device (7–12 MHz phased array scanner which was interfaced to an AGILENT SONOS 4500 ULTRASOUND MACHINE). Ultrasound study was performed by a single experienced radiologist who was blinded to the patients' clinical history. The common carotid artery (below the carotid bulb and 1cm proximal to bifurcation) was scanned with the neck in hyper-extension, on B mode (real-time) and Doppler imaging. The longitudinal section of the carotid artery was scanned and its wall was assessed for intimal thickness. The first line was the luminal-intimal interface, while the second was collagen-containing upper the layer of adventitia. CIMT was defined as the distance from the leading edge of the front echogenic line to the second hypoechoic line from the upper layer of the adventia. Both right and left common carotid arteries were scanned and the mean of both sides (recorded thrice) was taken as the common final value. The normal limit for CIMT is arbitrary and is influenced by age, gender and population. The definition of abnormal CIMT is less clearly defined in children. However, a value of >1 mm is definitely abnormal. All parameters were evaluated by a single experienced vascular sonographer who was blinded to the clinical and metabolic profile of the patients.

Statistical analysis

All statistical analyses were done using software version IBM SPSS (Statistical Package for the Social Sciences) statistics (Version 25.0, IBM Corp., USA, 2017–2018). Data were expressed descriptively as Mean±standard deviation (SD) for quantitative parametric data, median and interquartile range (IQR) for quantitative non-parametric values and as a percent for qualitative data. Comparative statistics were done using the independent student t-test for parametric data and the Mann-Whitney test for non-parametric data. Chi square test was used for comparison between two independent groups as regards the categorized data. Correlation analysis was performed using Pearson's correlation coefficient for parametric data and Spearman's rank correlation coefficient for non-parametric data. Odds ratio measures the association between an exposure and an outcome and how many times the risk was present among diseased individuals compared to that among non-diseased ones. In all statistical analyses, p-value <0.05 was considered significant.

Results

Endothelial progenitor cells (EPCs) number and the percentage was significantly lower in the patients' group (p=0.001) as shown in Figure 1. It was found that the mean percentage of EPCs was 1% (range 0.8–1.2) in control group compared to 0.46% (range 0.0.4–0.5) in the patients' group. Meanwhile, the mean CIMT in the patients' group was significantly higher in patients' group compared to control group (p=0.003) as shown in Figure 2. The mean of CIMT was 0.47 mm (range 0.45–0.50) in the control group compared to 0.58

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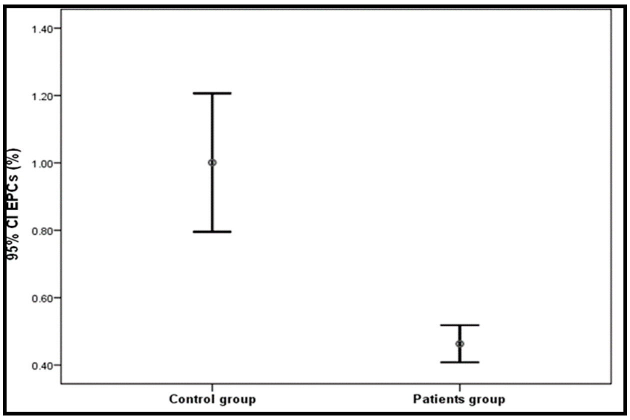

Figure 1: EPCs percentage comparison between patients with T1D and control group.

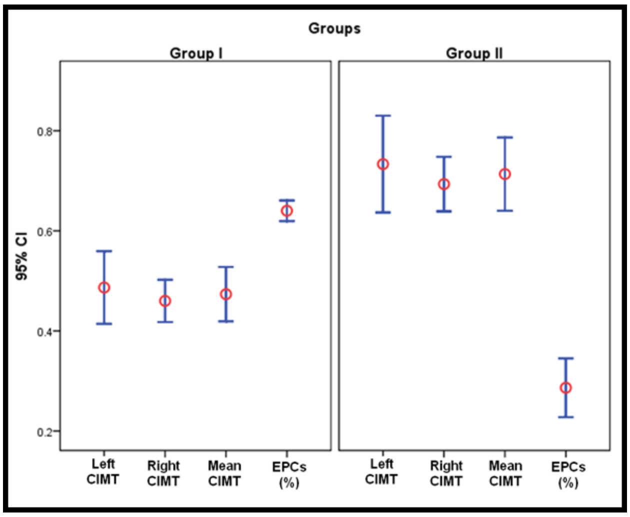

Figure 2: CIMT (mm) comparison between patients with T1D and control group.

mm (range 0.55–0.61) in the patients' group. Our study results also showed that the patients' group had significantly higher levels of total serum cholesterol (p=0.001) and LDL-cholesterol (0.015), and lower levels of HDL-cholesterol (p=0.003) than the control group.

The patients' group was subdivided further into 2 groups according to the HbA_{1c} levels; group I with HbA_{1c} less than 7.5 gm% and group II with HbA_{1c} more than 7.5 gm% as shown in Table 1. Group II patients had significantly higher mean random blood sugar (RBS) (p=0.002), total serum cholesterol (0.006), LDL cholesterol (0.033), and mean CIMT (p=0.001) while they had significantly lower EPCs percentage than control group as shown in Figure 3; EPCs and CIMT in both groups. Hypoglycemic attacks were more the common in group I than in group II (56.6% of

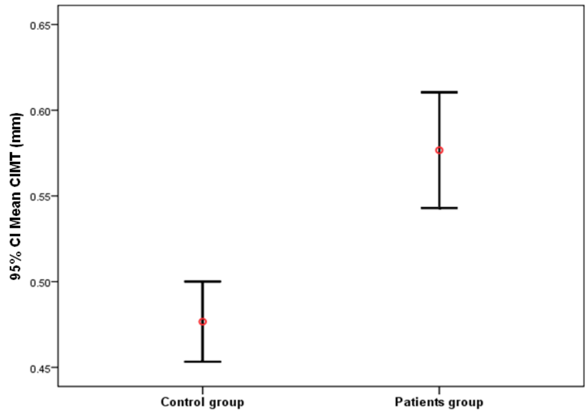

Figure 3: Comparison shows mean CIMT and EPCs% levels between group I and group II.

group I developed three or more attacks of hypoglycemia versus 30% of group II). DKA attacks were more common in group II (36.7% of group II patients developed 1–2 attacks versus 4.7% of group I in the last 6 months). About 2.6%, 43.3%, and 0% of group I patients developed diabetic retinopathy, diabetic nephropathy, and diabetic neuropathy respectively versus 10.2%, 15.7%, and 6.4%, respectively in group II patients.

There was a statistically significant negative correlation between EPCs percentage and mean random blood sugar (p=0.022), mean HbA_{1c}% (p=0.013), the presence of diabetic retinopathy (DR) (p=0.001) but not with diabetic nephropathy nor diabetic neuropathy, total serum cholesterol (0.003), LDL-cholesterol (p=0.015) and mean CIMT (p=0.021). Mean CIMT was positively correlated to duration of the disease (p=0.002), mean RBS (p=0.002), mean HbA_{1c}% (p=0.003), total serum cholesterol (p=0.017), triglycerides (p=0.029) and LDL-cholesterol (p=0.002) while it was negatively correlated to EPCs percentage (p=0.001) and HDL-cholesterol (p=0.016). Multi-regression analysis done for predictors of increased mean CIMT in the patients' group showed that EPCs% and HDL-cholesterol are significant predictors for increased CIMT in children and adolescents with T1D as shown in Table 2.

Discussion

Particular attention should be paid to the pre-clinical alterations in the vascular system in

TabIe 1: Comparison between group I and group II in patients with T1D regarding laboratory parameters and CIMT.

Parameters	Group I (HbA1c%<7.5%)		Group II (HbA1c%>7.5%)		p-Value
	Mean	SD	Mean	SD	
Mean RBS (mg/dl)	150.50	9.02	236.87	47.59	0.001
Micro albuminurea (μg/mg creatinine)					0.718
<30	26	86.70%	25	83.30%	
30–300	4	13.30%	5	16.70%	
Cholesterol (mg/dl)	153.47	31.11	173.53	34.62	0.018
Triglycerides(mg/dl)	93.05	30.01	89.67	27.07	0.649
LDL (mg/dl)	104.70	24.15	119.34	18.89	0.031
HDL (mg/dl)	42.99	6.90	40.86	8.28	0.284
Mean CIMT (mg/dl)	0.47	0.15	0.71	0.20	0.014
EPCs%	0.64	0.06	0.29	0.16	0.000

RBS: random blood sugar, LDL: low-density lipoproteins, HDL: high-density lipoproteins, CIMT: carotid intema media thickness, EPCs: endothelial progenitor cells.

Table 2: Multi-regression analysis for predictors of increased mean CIMT in patients group.

Independent variables	Coefficient	Std. error	p-Value
BMI (kg/m^2)	0.026	0.046	0.57
Mean RBS (mg/dl)	0.0072	0.003	0.05
Mean HbA_{1c}% over 1 year	–0.146	0.092	0.12
Cholesterol (mg/dl)	–0.0012	0.002	0.66
Triglycerides (mg/dl)	0.000091	0.001	0.95
LDL (mg/dl)	0.0012	0.003	0.74
HDL (mg/dl)	–0.011	0.004	0.007
EPCs%	–0.035	0.0021	0.006

BMI: body mass index, RBS; random blood sugar, LDL: low-density lipoproteins, HDL: high-density lipoproteins, CIMT: carotid intema media thickness, EPCs: endothelial progenitor cells.

children and adolescents with T1D in order to prevent the development of overt atherosclerosis and clinical events in adulthood. The high risk of atherosclerosis in diabetes is mainly attributed to endothelial dysfunction that results both from endothelial cell damage and impaired endothelial repair [14]. The present study was designed to estimate the level of EPCs as assessed by flow cytometric analysis of CD34/KDR and CD133 coexpression in children and adolescents with T1DM, and to study its relation to diabetes duration, metabolic control, macrovascular complications (as marked by CIMT) and microvascular complications. This study is aiming to be part of in the prediction and prevention of cardiovascular complications early in the disease. The microvascular complications of diabetes included diabetic retinopathy (DR), diabetic nephropathy (DN), and diabetic neuropathy and are related to damage to small vessels of the kidney, retina and nerves. Our results showed that 2.4% of all patients with T1D had retinopathy which goes with that of

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Shibeshi et al. who stated that the prevalence of diabetic retinopathy ranged from 0 to 28% in various studies [15]. In our study, it was found that patients with poor glycemic control and also patients with complications as DR had lower levels of EPCs. Other studies done in adults with T1D have shown that the number of EPCs is correlated to the presence of diabetic complications. Asnaghi et al. found that EPCs is increased in T1D patients with proliferative DR [16]. Meanwhile, Brunner S. et al., concluded that in patients with T1D with diabetic retinopathy, EPCs undergo stage-related regulation; in non-proliferative retinopathy, a reduction of EPCs was observed, while in proliferative DR, a dramatic increase of mature EPCs was observed [17].

In the current study, when comparing optimally controlled group I to sub-optimally controlled group II we found that microvascular complications are definitely more in group II. Similarly, the prevalence of DN among children and adolescents with T1D varies much between different studies. These results show a significant relationship between the degree of glycemic control and the occurrence of nephropathy and are in agreement with the epidemiological analysis of the Diabetes Control and Complications Trial (DCCT) and the United Kingdom Prospective Diabetes Study (UKPDS) where a curvilinear relationship between HbA_{1c} and microvascular complication was demonstrated [18]. However, less developed countries reported higher figures; a study in Iraq, MA (microalbuminurea) occurred in 41.4% of patients whereas in an Iranian study MA occurred in 34.6% of 81 children and adolescents with diabetes [19]. Our study showed that 30% of all our patients had diabetic nephropathy.

There is considerable uncertainty as to the prevalence of diabetic neuropathy in the pediatric age group, and this is probably due to the lack of large epidemiological studies performed in pediatric patients with diabetes, who often show few symptoms of neurological involvement; in studies with comprehensive epidemiological data involving both adult and pediatric patients, they showed that the percentage of peripheral neuropathy ranged from 7% to 57% [20]. This wide difference among studies may be due to different diagnostic criteria and to the lack of clear and appropriate diagnostic criteria for diabetic neuropathy. However, a study with the most complete electrophysiological evaluation reported a prevalence of 57% of subclinical neuropathy in children and adolescents with T1D [21]. Poor glycemic control is closely linked to the development of complications. The Diabetes Control and Complications Trial (DCCT) and the Epidemiology of Diabetes Interventions and Complications (EDIC) have clearly shown the role of strict glycemic control in reducing the risk of microvascular complications in subjects with T1D. In the adolescent cohort of the DCCT, a positive effect of improved glycemic control on complication risk was obtained. Most studies cited by the consensus review committees found that microvascular complications develop around an average of a decade after diagnosis thereafter the prevalence increases steadily with time [22].

The American Heart Association categorized children with T1D as the highest risk for cardiovascular risk and recommends both lifestyle and pharmacological treatment for those with elevated LDL cholesterol levels [23]. Atherosclerotic vascular disease in diabetes occurs continuously in children and adolescents with T1D as it overts much earlier than in healthy subjects later in adulthood. Cardiovascular risk factors that are typically associate with T1D, such as hypertension, obesity and dyslipidemia concur with the accelerated risk of cardiovascular disease [24]. Our patients had abnormal lipid profile levels; they had significantly higher levels of cholesterol, triglycerides, and LDL cholesterol and significantly lower levels of HDL cholesterol compared to healthy controls. These results agreed with the results of Al-Naama et al., who studied lipid profiles in 32 diabetic children over a period of 1 year and found that total cholesterol, triglycerides, LDL-cholesterol, VLDL-cholesterol were significantly higher in diabetic children compared to the control group, however, HDL-cholesterol was also higher in diabetic children with T1D in their study compared to healthy controls [25]. Moreover, a study conducted a cross-sectional analysis of 512 youth with T1D and 188 healthy control subjects aged 10–22 years in Colorado and South Carolina. Fasting lipid profiles were compared between youth with T1D (stratified according to categories of optimal, HbA_{1c}% <7.5%, and suboptimal, HbA_{1c}% ≥7.5%, glycemic control) and healthy non-diabetic youth, using multiple linear and logistic regression they found that youth with T1D and optimal HbA_{1c}% had lipid concentrations that were similar; total cholesterol, LDL-cholesterol, and LDL particle size or even less atherogenic than those observed in non-diabetic youth; HDL-cholesterol, non-HDL-cholesterol, triglycerides, and triglyceride to HDL-cholesterol ratio, whereas youth with suboptimal glycemic control had elevated standard lipid levels; total cholesterol, LDL-cholesterol and non-HDL-cholesterol [26]. These results agreed with our results as regards comparison to controls. However, our patients in group I (HbA_{1c}% <7.5) still had higher levels of total serum cholesterol, triglycerides and LDL-cholesterol and lower levels of HDL-cholesterol than healthy controls. Low levels of HDL-cholesterol in poorly controlled patients with T1D could be attributed to being part of dyslipidemia which occurs in the course of the disease where HDL-cholesterol is dysfunctional and sometimes decreased in serum [38]. The pathogenesis of endothelial dysfunction in T1D is complex and involves several mechanisms such as inflammation, oxidative stress, the interaction between insulin and C peptide and most importantly decreased number of EPCs [27].

We found that EPCs percentage is significantly reduced in patients compared to controls. Also when comparing optimally controlled group I to sub-optimally controlled group II. These findings go with that of Sibal et al. who studied circulating EPCs, they found a significant reduction in circulating cell counts of some populations of both classes of cells (endothelial progenitor, circulating progenitor cell subtypes) in young people (mean age 25±5 years) with T1D without any macrovascular disease or microalbuminuria in their study they compared EPCs number in T1D young people with that of healthy controls and they did not correlate EPCs number to the degree of diabetic control, optimal (HbA_{1c}%<7.5) and suboptimal (HbA_{1c}%>7.5) [12]. Indeed we found that EPCs% was inversely related to HbA_{1c}% supporting the results of Cindy et al., who studied EPCs number in 20 patients with T1D (mean age 39±14 years) and 20 healthy matched controls and found that EPCs number is significantly decreased in diabetic patients and it is inversely related to HbA_{1c}% level [28]. Also, Di Meglio et al. reported decreased levels of circulating EPCs delineated by CD34+CD133+CD31C phenotype in a slightly younger (18–22 years) small group of type 1 diabetic patients. So far, only a few studies have been conducted in children with present cardiovascular risk factors to examine whether a reduction of EPCs is present at early stages of the disease and whether alterations in circulating progenitor subpopulations correlate with endothelial function [29].

Cardiovascular disease as a result of macrovascular atherosclerotic changes is the major cause of mortality among patients with diabetes mellitus. Even if these complications affect predominantly adult diabetic patients, the process of vascular changes starts much earlier [30]. Autopsies have shown that the atherosclerotic processes at the endothelial level begin in childhood and progress rapidly in the presence of risk factors [31]. Thus, children with diabetes mellitus are considered as high-risk patients and special attention to vascular health has been recommended. CIMT is an accepted predictor for future cardiovascular events independent of age, gender and cardiovascular risk factors. From childhood to early adulthood CIMT is the only atherosclerotic marker of the carotid tree; plaques occur later in life. Both parameters contribute independently to risk assessment for future cardiovascular events [32].

Available epidemiological data showed that CIMT is significantly correlated with future cardiovascular events. It is particularly useful in individuals classified as being at intermediate or high risk by the presence of multiple conventional risk factors. CIMT is such a marker that can be used to identify the subclinical atherosclerotic disease [33]. Sibal et al. found that CIMT increased significantly in patients with T1D, however, they did not find any relationship between CIMT and EPCs in the T1D group [12]. Our study agreed with their results in the part that CIMT increased significantly in diabetic patients but we found also that EPCs% is correlated to CIMT with an inverse relationship. Carlo et al. compared 16 young patients with T1D free of overt

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clinical complications with 26 healthy controls of similar age, and found a significantly reduced number of specific $CD34^{+}KDR^{+}$ EPCs in patients group and, in the entire study population, EPCs count were inversely and independently related to CIMT [34]. Gian et al. evaluated whether variations in the number of EPCs are associated with subclinical atherosclerosis in healthy non-diabetic subjects and found that, CD34 KDR endothelial progenitor cells were inversely correlated with CIMT, even after adjustment for hs-CRP and 10-year Framingham risk and independently of other cardiovascular parameters [35].

Rocio et al. also compared the CIMT in Hispanic pediatric T1D patients against that in healthy control subjects matched for age, sex, height, and BMI, they found that type 1 DM is associated with higher CIMT and decreased flow velocities in a Hispanic pediatric population. Masoud et al. conducted a case-control study to compare CIMT in 40 diabetic children with 40 healthy matched controls and they found that CIMT was significantly higher in the diabetic group. Ashima et al. also found that mean CIMT was higher in patients with higher HbA_{1c}% versus those with normal HbA_{1c}% in their study conducted on 30 diabetic patients with a range of age 10–18 years [36].

Declaration

All authors declare that there is no financial or institutional conflict of interest. This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sector.

Conflict of interest

The authors declare no conflict of interest.

Conclusion

EPCs levels are significantly reduced in children and adolescents with T1D and are correlated negatively to mean random blood sugar, HbA_{1c}%, cholesterol, LDL-cholesterol, and mean CIMT. It was found that EPCs% is a good predictor for increased CIMT in children with T1D. However, longitudinal studies are needed to confirm this relationship. Children and adolescents with T1D are at increased risk for atherosclerosis and macrovascular complications marked by lower EPCs, increased levels of harmful lipids; total cholesterol, triglycerides, LDL-cholesterol, and increased mean CIMT. This may contribute to new strategies for early detection and intervention to prevent macrovascular complications. Larger prospective studies are needed to confirm our results. Constructed follow-up for glycemic control and lipid profile should be planned for each diabetic patient. EPCs levels can be used as a suggestive marker for macrovascular complications. CIMT may be included in the routine follow-up of diabetic patients at high risk for atherosclerosis; high blood pressure and dyslipidemia.

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