



Uncontrolled Type 1 Diabetes Mellitus and Endothelial Dysfunction in Adolescents

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Abstract

Background: Diabetes mellitus is associated with microvascular and macrovascular diseases, potentially manifested as endothelial dysfunction. In adults with type 2 diabetes the haptoglobin genotype 1-1 has been shown to have a protective role in inhibiting the development of complications. Although complications from type 1 diabetes are infrequent during childhood, endothelial dysfunction, which is an early marker of vascular complications, may occur.

Objectives: To evaluate endothelial function in adolescents with type 1 diabetes before the development of complications and to test for potential relationships between endothelial dysfunction and haptoglobin genotype.

Methods: The study group comprised 15 adolescents with type 1 diabetes. All underwent a general physical examination, diabetes control evaluation (including HbA1c levels), endothelial function assessment and haptoglobin genotype determination.

Results: There was a significant negative correlation between HbA1c levels and endothelial function ($r = -0.48$, $P < 0.05$), and HbA1c was significantly higher in patients with endothelial dysfunction than in those with normal endothelial function (9.9 ± 2.2 vs. 7.7 ± 1.0 mg/dl, $P < 0.05$). In addition, there was a tendency toward a positive correlation between high density lipoprotein and endothelial function ($r = 0.4$, $P < 0.1$). There was no correlation between the haptoglobin genotype and endothelial function.

Conclusions: These results show that even in patients without complications, uncontrolled type 1 diabetes is associated with endothelial dysfunction, which may lead to microvascular complications in the future.

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Macrovascular and microvascular diseases are currently the principal causes of morbidity and mortality in patients with type 1 and type 2 diabetes mellitus [1]. Loss of the modulatory role of the endothelium may be a critical and initiating factor in the development of diabetic vascular disease [2]. Endothelial cells are responsible for controlling blood vessel tonus and its response to physiological and pathological stimuli, and for the release of vasodilating agents (nitric oxide, prostacyclin, endothelial derived

hyperpolarizing factor). Therefore, the functioning endothelium keeps the blood vessels intact by releasing anticoagulating, anti-inflammatory, fibrinolytic and vasodilating agents in order to save the blood vessels from destructive oxidative stress. Endothelial dysfunction is defined as impaired vasodilation in response to acetylcholine or hyperemia (both induce nitric oxide-dependent vasodilation) [1]. Endothelial function, defined as an increase in blood vessel diameter or flow in response to appropriate stimuli, can be measured by physiological, mechanical or biochemical means [2,3]. Quantitative coronary angiography is considered the gold standard method for assessing endothelial dysfunction, but this method is invasive and expensive, and is available in only a few centers. In addition, it requires expertise and poses risks. However, there are two alternative non-invasive methods: brachial artery ultrasound scanning, and peripheral arterial tonometry. With the first method, ultrasonography is used to measure the change in the brachial artery diameter in response to the release of the arterial occlusion, which typically lasts 5 minutes. The advantages of this method are its simplicity and its non-invasive nature. However, it does have drawbacks: the results are operator dependent and there are no corrections for potential systemic autonomic nervous system activation [3,4].

Genetic factors seem to play a role in the tendency of diabetic patients to develop atherosclerosis. The haptoglobin gene is one such factor [5]. Haptoglobin is a serum protein that has two alleles (1 and 2). The genotype (chromosome 16) can be homozygous for 1 (Hp 1-1), homozygous for 2 (Hp 2-2) or heterozygous (Hp 1-2). These alleles are differently distributed around the world. In Israel, 9% of the population are 1-1, 49% are 2-2 and 42% are 1-2 (as in the United States and Europe). The distribution among diabetic patients is similar to that in the general population [6]. Haptoglobin is an antioxidant, probably stabilizing the heme in the hemoglobin. The ability of the haptoglobin to protect the heme from oxidative stress can be damaged during the process of hemoglobin glycosylation, as seen in diabetes [5]. It was recently demonstrated that diabetic indi-

Hp = haptoglobin

viduals who are homozygous for haptoglobin 1 are less affected by oxidative stress than those who are homozygous to allele 2, as their glycosylated hemoglobin is eliminated by macrophages [7]. Indeed, studies have shown that the haptoglobin gene is an independent risk factor for coronary heart disease in patients with type 2 diabetes mellitus. In addition, it was found that an antioxidant agent such as vitamin E can be helpful in patients with haptoglobin 2-2. These studies were performed on adult type 2 diabetic patients; studies on children with type 1 diabetes are sparse [8-11].

In the current study we sought to examine the association between diabetic profile, haptoglobin genotype and endothelial function among adolescents with type 1 diabetes prior to the development of any complication. We hypothesized that endothelial dysfunction will correlate with longer duration and poorer control of diabetes. We further hypothesized that haptoglobin 1-1 will protect diabetics from developing this disorder.

Patients and Methods

Fifteen patients, age 14–20 years, with type 1 diabetes of 2 months to 10 years duration (4.1 ± 2.6 years) were recruited from the diabetic clinic at Meyer Children's Hospital, Rambam Medical Center. Ophthalmology follow-up and creatinine level were normal in all patients and none had microalbuminuria; blood pressure was normal and there were no signs or presumed symptoms of peripheral neuropathy. Exclusion criteria included a complication of diabetes (diabetic retinopathy, abnormal kidney function, hypertension, or neuropathy). Patients who were included in the study were treated solely with insulin and with no other medication, and none of them were smokers. The study was approved by the Rambam Institutional Review Board, and patients (or their parents, depending on the patient's age) gave their signed informed consent prior to participation (patients < 18 years old agreed to participate but their parents signed the consent form). Anthropometric and physical data for each participant were taken from the medical charts or were recorded by the investigators during the test (height, weight, etc.). Levels of high density lipoprotein and low density lipoprotein as well as total cholesterol and triglycerides were available for each patient during the month preceding the study. HbA1c levels were measured during the first week of the study. In addition, on the first day of the study, prior to studying endothelial function, we measured the blood glucose level (using Elite, a commercial glucometer). The test was done in the morning (8:00–10:00) and the participants were asked to fast for at least 3 hours before the test. The blood glucose level range was 80–180 mg/dl (those with a lower glucose level had to eat or drink, and those with a higher level were excluded in order to prevent the influence of hyperglycemia). In each participant endothelial function was assessed by means of peripheral arterial tonometry, after which a blood sample for the haptoglobin phenotype was taken

PAT = peripheral arterial tonometry

HDL = high density lipoprotein

Assessment of endothelial function

Endothelial function was assessed utilizing the ENDO-PAT200 device (Itamar Medical Ltd., Caesarea, Israel). This method has been previously described in detail [12]. Briefly, it is a non-invasive technique that analyzes endothelial function by reactive hyperemia in response to arterial occlusion, using a pneumatic probe attached to the subject's second finger. The pulse wave amplitude changes in the digital arteries are assessed following 5 minutes of brachial arterial obstruction (inflating a blood pressure cuff to 50 mmHg above systolic pressure), using a special finger plethysmograph. The results from the tested arm are corrected for potential systemic changes, based on the pulse wave amplitude from the non-tested (control) arm. A decrease (vasoconstriction) or lack of increase (vasodilation) indicates endothelial dysfunction. Based on data from our laboratory and the manufacturer's operating manual, the normal threshold stands at 1.67 for young adults [11,13]. There are only a few reports regarding the normal endo-PAT threshold for children and adolescents [14], suggesting that this threshold value may apply for adolescents as well. Therefore, in the current study we adopted these reference values and considered the normal threshold as 1.67. All tests were performed by the same investigator who was not blinded to the clinical data.

Data analysis

Statistical analysis included comparisons between biochemical values and endothelial function in patients with various disease durations, as well as between patients with normal or impaired endothelial function. In addition, correlation analyses were performed between the indices of diabetes and endothelial dysfunction. The results are expressed as average \pm standard deviation. The statistical test used was the two-tailed *t*-test, with $P < 0.05$ considered statistically significant.

Results

Nine of the 15 study patients were males (60%). Table 1 summarizes the comparisons between the patients with impaired and normal endothelial function. There were no differences in age, gender, duration of illness, body mass index, blood pressure, triglycerides or cholesterol between the two groups. A significantly higher level of HbA1c was found in the endothelial dysfunction group compared to the normal endothelial function group. Furthermore, there was a significant negative correlation between HbA1c and endothelial function ($r = -0.48$, $P < 0.05$) [Figure 1].

Interestingly, with increasing age the endothelial function was better ($r = 0.47$, $P < 0.05$). In addition, there was a tendency (although not statistically significant) for a positive correlation between HDL and endothelial function ($r = 0.4$, $P < 0.1$). There was no correlation between the haptoglobin genotype and endothelial function [Table 2].

Discussion

This study demonstrates that in a group of adolescents with type 1 diabetes mellitus, uncontrolled diabetes (manifested by higher

Table 1. Comparison between patients with normal and impaired endothelial function

Parameter	All participants (n=15)	Normal endothelial function (A) (n=8)	Impaired endothelial function (B) (n=7)	P value (comparing A and B) NS
Age (yrs)	17.4 ± 1.7	18 ± 1.25	16.67 ± 1.87	0.140
Duration of illness (yrs)	4.1 ± 2.6	4 ± 2.93	4.1 ± 2.38	0.963
Body mass index	22.2 ± 2.6	21.82 ± 2.84	22.69 ± 2.46	0.535
Systolic blood pressure (mmHg)	117 ± 12	116 ± 14.25	117.71 ± 9.27	0.785
Diastolic blood pressure (mmHg)	62 ± 6	60.25 ± 6.71	63.86 ± 4.38	0.236
HbA1c (%)	8.7 ± 2	7.66 ± 1	9.89 ± 2.24	0.042
Triglycerides (mg/dl)	77 ± 36.4	71.86 ± 35.31	83 ± 40.05	0.609
Total cholesterol (mg/dl)	171.8 ± 35.3	161.73 ± 15.37	180.43 ± 45.84	0.342
HDL (mg/dl)	56.5 ± 16.4	60.57 ± 18.54	52.47 ± 14.13	0.191
LDL (mg/dl)	100 ± 30	88.86 ± 8.82	111.47 ± 40.13	0.377
Fasting glucose (mg/dl)	145 ± 25	137.88 ± 27.6	152.86 ± 21.87	0.263
Endothelial function (PAT score)	1.68 ± 0.5	2 ± 0.46	1.33 ± 0.16	0.004

Data in the table represent average ± standard deviation

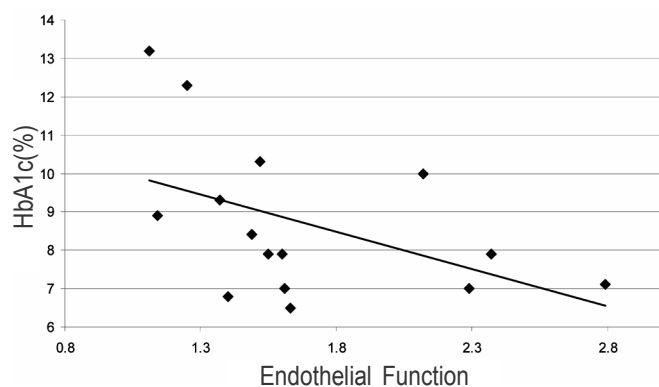


Figure 1. Correlation between HbA1c and endothelial function. Note that as HbA1c increases so the endothelial function index decreases (indicating worse function). The correlation coefficient is $r = -0.48$, $P < 0.05$.

Table 2. Endothelial function in various haptoglobin genotypes

Haptoglobin genotype	Normal endothelial function	Endothelial dysfunction
1-1	2	1
2-1	1	2
2-2	5	4

Numbers indicate the patients in each category

HbA1c) is associated with endothelial dysfunction. Reduced HDL also seems to relate to endothelial dysfunction, although to a lesser extent. Surprisingly, we did not find a protective effect of the haptoglobin genotype 1-1, which may be related to the small number of patients.

The association between uncontrolled diabetes and endothelial dysfunction is not surprising. Hyperglycemia is well known for its influence on the pathogenesis of diabetic complications.

Hyperglycemia induces acute changes in intracellular metabolism, as well as long-term changes in macromolecules through the formation of advanced glycation end-products. All pathways lead to oxidative stress, which is followed by endothelial damage [1]. A close relationship between endothelial dysfunction and metabolic control was found in streptozocin-diabetic rats in which the degree of hyperglycemia was manipulated with subcutaneous insulin implants [1]. In addition, another research showed that metabolic control and HbA1c levels were unbalanced and high in cases of endothelial dysfunction [15]. However, other studies did not find such a relationship between glucose levels, HbA1c, metabolic control and endothelial dysfunction [16-18]. The reason for the inconsistent reports is not clear at this time. There may

be some important factors that modulate the effect of glucose and HbA1c on endothelial function. Indeed, it was shown in adults that the haptoglobin genotype 1-1 may protect against cardiovascular complications of diabetes (and thus probably also against endothelial dysfunction) [5]. In our study we did not find such an effect, but the current study was probably under-powered to show such an effect. Since the prevalence of haptoglobin 1-1 in the population is relatively low, large numbers of participants are necessary to determine the influence of this serum protein. In addition, the association between endothelial dysfunction and diabetes should be assessed differently in adults because many type 2 diabetic patients have several risk factors, and there are also some differences in the pathogenesis of the complications of diabetes. Therefore, comparing studies of adults with type 2 diabetics to those of type 1 diabetic children is problematic.

The correlation we found between age and endothelial function is somewhat surprising. There are insufficient data on normal endothelial function during childhood and adolescents and the effects of growth on this functioning. These results may be related to the smaller finger diameter of children compared to adults. While the method we used is well reported in adults [8,12,13,19-21], the experience in children is sparse. Additional studies and a long-term follow-up of the participants from all previous studies will probably shed light on this unclear finding.

Another observation in our study was the tendency toward correlation between lipid profile and endothelial function. The endothelial function improved as the levels of HDL increased and the levels of LDL decreased (albeit not significant). These relationships could be expected and are in concert with many previous publications stressing the importance of lipid profile in assessment of cardiovascular risks. HDL has several roles: removing cholesterol from the blood vessel, maintaining endothelial cell integrity, reducing platelet aggregation and coagulation and

LDL = low density lipoprotein

potentiating vasodilation. Indeed, improved endothelial function with increasing HDL level is well established [22-25].

Our study has several limitations. The main limitation was the relatively small sample size. Due to difficulties in recruiting patients and especially in the light of strict inclusion and exclusion criteria (fasting 3 hours prior to the study, glucose levels of 60–180 mg/dl, etc.), we did not succeed in recruiting a large-enough group within a reasonable time. We believe the small sample size mostly affected the investigation for the effect of haptoglobin genotype. Yet, the statistically significant effect of HbA1c despite the small sample size emphasizes the importance of this finding. The second limitation was the lack of a control group which could have helped in interpreting the findings. This was due to ethical issues and difficulties in recruiting healthy children for a study involving blood sample drawing and endothelial function assessment. Third, the current study assessed endothelial function by response to ischemia-related hyperemia, but it did not document the potential for vasodilation due to non-endothelial related mechanisms (i.e., direct smooth muscle relaxation by nitroglycerin). Therefore, there may be a ceiling effect on our results, although this is unlikely.

Despite these limitations, we believe our findings are important and further support the previously reported requirement for tight control of blood glucose levels. Moreover, it is tempting to recommend the routine use of endothelial function tests for early detection of diabetes complications. Obviously, additional large-scale studies with control groups are warranted.

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