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Dysfunction of the Vascular Endothelium in Egyptian Children with Insulin-Dependent Diabetes Mellitus

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The aim of the present study was to assess plasma endothelin-1 (ET-1) and Nitric Oxide (NO) levels in children with Insulin-dependent Diabetes Mellitus (IDDM) and their relation to the degree of metabolic control and disease duration. The study group consisted of 34 children with IDDM and 17 healthy controls of matched age and sex, recruited from the Pediatric Endocrinology and Diabetes Unit; Mansoura University Children's Hospital, Egypt, during the period January to March, 2005. Plasma ET-1 and NO levels were assessed by enzyme immunoassay and we evaluated their possible relation with metabolic control and disease duration. Plasma ET-1 levels were significantly higher in diabetic patients compared to controls, ($p = 0.02$). Patients with poor or moderate metabolic control had significantly higher levels of ET-1 compared to those with ideal control ($p = 0.004$ and 0.001), respectively. A +ve significant correlation was found between plasma ET-1 levels and NO, HbA1c levels and disease duration, ($p = 0.004$, 0.001 and 0.02), respectively. Although plasma NO levels in diabetic patients were not significantly different from controls, yet they were significantly higher in patients with poor metabolic control compared to those with ideal control ($p<0.001$). In children with IDDM, poor metabolic control and increased disease duration are associated with increased ET-1 production, which may be related to future diabetic complications. The elevated plasma NO levels in poorly controlled patients may suggest a compensatory protective response towards increased ET-1 production.

Key words: Endothelin-1, nitric oxide, IDDM, children, type-1 diabetes, endothelial dysfunction

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INTRODUCTION

Diabetes Mellitus (DM) is a chronic metabolic disorder caused by an absolute or relative deficiency of insulin, an anabolic hormone, leading to type 1 diabetes [Insulin Dependent Diabetes Mellitus (IDDM)] (William, 2005). It commonly occurs in childhood and is an important cause of morbidity and mortality among young people (Scott *et al*., 2004; Lane, 2005). Despite modern insulin treatment, >50% of patients with childhood-onset type 1 diabetes developed detectable diabetes complications after approximately 12 years of onset (Svensson *et al*., 2004).

Complications of diabetes include retinopathy, nephropathy, neuropathy, macrovascular disease and associated autoimmune diseases. Clinical manifestations of complications uncommonly present in childhood and adolescence (Glastras *et al*., 2005).

The endothelium which is a primary target of unbalanced glycaemic control is involved in the pathogenesis of vascular complications in patients with type-1 diabetes mellitus (DM). Affected children have early endothelial dysfunction which is defined as the loss of endothelium properties, e.g., alteration of protein synthesis, increased vascular tone and permeability, acquisition of prothrombotic and antifibrinolytic properties (Wiltshire *et al*., 2002; Pomilio *et al*., 2002).

The endothelium plays an important role in vascular homeostasis by synthesizing and releasing substances that modulate vascular tone and structure, as well as the interactions of circulating cells with the vessel wall (Cardillo *et al*., 2002).

Endithelin-1 (ET-1) is the substance potentially involved in the vasomotor deregulation of patients with diabetes, as well as the development of their vascular complications (Sarman *et al*., 1998; Hopfner and Gopalakrishnan, 1999). ET-1 is a 21-amino-acid peptide produced by ischaemic or injured vascular endothelial cells (Bek *et al*., 2002; Glowinska *et al*., 2004). It exerts its vascular effects via specific binding to 2 receptor subtypes, ET_A and ET_B. On vascular smooth muscle cells, both ET_A and ET_B receptors mediate vasoconstriction (Seo *et al*., 1994; Haynes *et al*., 1995), whereas ET_B receptors on endothelial cells cause vasodilatation, predominantly because of activation of the L-arginine-nitric oxide pathway (NO) (Verhaar *et al*., 1998). Other effects of ET-1 include the activation of smooth muscle cell mitogenesis, leukocyte adhesion and monocyte chemotaxis, thereby implying a potential involvement of this peptide in the initiation and/or the progression of the atherosclerotic process (Cardillo *et al*., 2002).

An elevated plasma ET-1 level, which is a strong vasoconstricting agent, has been reported in diabetic patients (Bruno *et al*., 2002; Glowinska *et al*., 2004). This study was carried out to evaluate ET-1 and NO as markers of endothelial activation in children with IDDM and to clarify their relation to the degree of metabolic control and to the disease duration.

MATERIALS AND METHODS

The study includes 34 children with IDDM recruited from the Pediatric Endocrinology and Diabetes Unit; Mansoura University Children's Hospital, Egypt during the period January to March, 2005. They were 13 male and 21 female. Their ages ranged from 4 to 14 year [median (IQR) = 11 (8-12) year]. The protocol was approved by the ethics committee in the National Research Center and consent was obtained from each child's parents.

Patients were diagnosed according to the WHO diagnostic criteria (Puavilai *et al*., 1999). Patients were treated with two or more daily doses of insulin (Subcutaneous injection) and their diet and exercise were supervised. Their disease duration ranged from 2.5 up to 7 years [median (IQR) = 3 (2-5) year]. Ten patients had disease duration >4 years and 24 had disease duration <4 years. All patients had normal fundus examination and normal kidney function with no urinary evidence suggestive of diabetic nephropathy. Only two patients had systolic and diastolic blood pressure values between 90th and 95th centile for age and the remaining patients had normal blood pressure levels (Zahra, 1987). Patients were classified according to the guidelines of the International Society of Pediatric and Adolescent Diabetes for metabolic control into 3 subgroups (A 25th Annual meeting of the International Society for Pediatric and Adolescent Diabetes, 1999); Patients with ideal control (HbA1c<7.6%) (n = 17), patients with moderate control (HbA1c = 7.6-9%) (n = 9) and patients with poor metabolic control (HbA1c > 9%) (n = 8). A group of 17 healthy children of matched age and sex were used as a control group.

The plasma ET-1 levels were assayed by the qualitative enzyme immunoassay technique kit (R and D system inc., USA) (Porstmann and Kiessig, 1992). This technique depends on a reaction between an antibody specific for ET-1 precoated onto a microplate and ET-1 present in the sample. The minimum detectable level for the assay is 1.0 pg mL^{-1}. The plasma concentrations of NO were assayed by enzyme immunoassay kit (R and D system inc., USA) (Hegesh and Shiloah, 1982). This technique determines the total NO based on the enzymatic conversion of nitrate to nitrite by nitrate reductase enzyme. The level of nitrite is assayed by colorimetric

assay. The detection limit for assay for NO is 1.0 μmol L^{-1}. HbA1c level was assayed by the quantitative colorimetric kit (Stabinow Laboratory, USA) (Grigorov *et al.*, 1984).

Statistical analysis: The data of the study were analyzed by SPSS under windows (version 10). Data were found to be non-parametric by Kolmogorov Smirnov test. Man-Whitney U test was used to compare plasma ET-1 and NO levels in the different groups. Spearman correlation coefficient was used for correlation between the quantitative variables. A p-value<0.05 was considered significant.

RESULTS

Diabetic patients had significantly higher plasma ET-1 levels when compared to controls [median (IQR) = 5.9 (4.9-39.2 Vs 4.9 (4.4-6.1) pg mL^{-1}, respectively; p = 0.02] (Table 1).

Patients with poor metabolic control had significantly higher ET-1 levels when compared to those with ideal control [median (IQR) = 39.8 (7.4-44.4) Vs 5.0 (4.6-5.7) pg mL^{-1}, respectively; p = 0.004). Similarly patients with moderate metabolic control had elevated ET-1 levels when compared to those with ideal control [median (IQR) = 24.0 (7.5-38.1) Vs 5.0 (4.6-5.7) pg mL^{-1}, respectively; p = 0.001 (Table 2).

Patients with disease duration >4 years had significantly higher ET-1 levels when compared to those with disease duration < 4 years [median (IQR) = 25.8 (12.3-38.1) Vs 16.4 (5.6-20.2) pg mL^{-1}, respectively; p<0.0001 (Table 3).

ET-1 levels correlated positively with NO levels (r = 0.48, p = 0.004), HbA1c levels (r = 0.57, p = 0.001) and disease duration (r = 0.39, p = 0.02) (Fig. 1-3).

Table 1: Plasma Endothelin-1 and Nitric oxide levels in IDDM patients and control

Parameter	IDDM (n = 34)	Control (n = 17)	p*
Endothelin-1 (pg mL^{-1})	5.9 (4.9-39.2)	4.9 (4.4-6.1)	0.02
Nitric oxide (μmol L^{-1})	24.65 (21.9-30.2)	22 (21-26.5)	0.09

Values expressed as median (IQR); *Mann-Whitney-U test

Table 2: Plasma Endothelin-1 and Nitric oxide levels in IDDM patients according to the degree of metabolic control

Parameters	Ideal control (n = 17)	Moderate control (n = 9)	Poor control (n = 8)	p1*	p2*
Endothelin-1 (pg mL^{-1})	5.0 (4.6-5.7)	24.0 (7.5-38.1)	39.8 (7.4-44.4)	0.004	0.001
Nitric oxide (μmol L^{-1})	22 (20.6-22.8)	24.5 (22.7-31.5)	31 (27.6-33)	<0.001	0.08

Values expressed as median (IQR); *Mann-Whitney-U test, p1 = Poor control Vs Ideal control; p2 = Moderate control Vs Ideal control

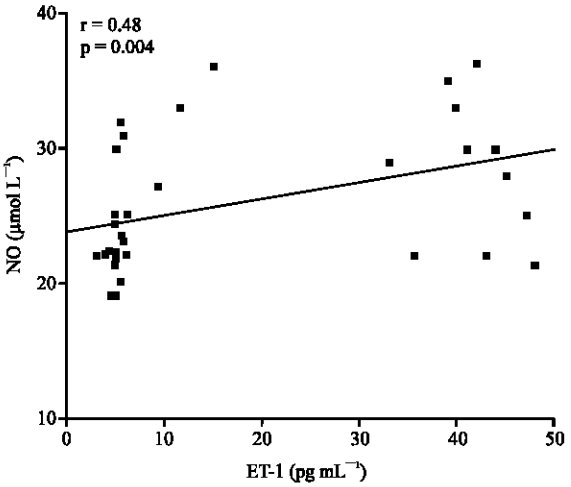

Fig. 1: Correlation between plasma endothelin-1 (ET-1) and nitric oxide (NO)

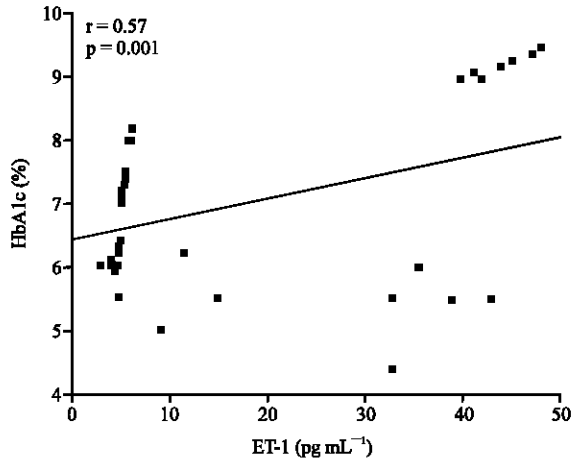

Fig. 2: Correlation between plasma endothelin-1 (ET-1) and HbA1c

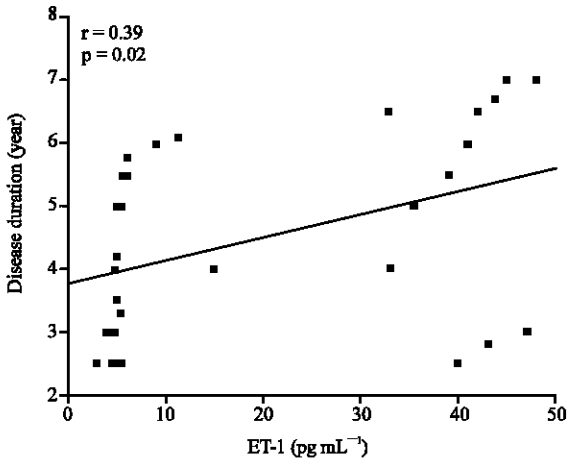

Fig. 3: Correlation between plasma endothelin-1 (ET-1) and disease duration

Table 3: Plasma Endothelin-1 and Nitric oxide levels in IDDM patients according to disease duration

Parameters	Duration > 4 years (n = 20)	Duration < 4 years (n = 14)	p*
Endothelin-1 (pg mL^{-1})	25.8 (12.3-38.1)	16.4 (5.6-20.2)	<0.0001
Nitric oxide (μmol L^{-1})	23.7 (22.1-24.6)	25.9 (21.9-29.2)	0.066

Values expressed as median (IQR); *Mann-Whitney-U test

Table 4: Correlation between plasma Nitric oxide level and HbA1c and disease duration

	Nitric oxide	
	r*	p
HbA1c	0.41	0.10
Disease duration	0.33	0.09

Spearman's correlation coefficient

Plasma NO levels in diabetic patients were not significantly different from controls [median (IQR) = 24.65 (21.9-30.2) Vs 22.0 (21.0-26.5) μmol L^{-1}, respectively; p = 0.09] (Table 1). Patients with poor metabolic control had significantly higher NO levels when compared to those with ideal control [median (IQR) = 31.0 (27.6-33.0) Vs 22.0 (20.6-22.8) μmol L^{-1}, respectively; p < 0.001). NO levels in patients with moderate metabolic control were not significantly different from those with ideal control [median (IQR) = 24.5 (22.7-31.5) Vs 22.0 (20.6-22.8) μmol L^{-1}, respectively; p = 0.08) (Table 2). A non- significant relation was found between NO levels and either of HbA1c levels or disease duration [(r = 0.41, p = 0.1), (r = 0.33, p = 0.09), respectively] (Table 4).

DISCUSSION

Several mechanisms of endothelial dysfunction in diabetes have been suspected; including impaired signal transduction, or substrate availability, impaired release of endothelium derived releasing factors (EDRF), increased destruction of EDRF, enhanced released of endothelium-derived constricting factors and decreased sensitivity of the vascular smooth muscle to EDRF (DeVriese *et al.*, 2000).

The present study revealed that plasma ET-1 is significantly elevated in diabetic children compared to controls [median (IQR) = 5.9 (4.9-39.2) Vs 4.9 (4.4-6.1) pg mL^{-1}, p = 0.02]. Similarly Glowinska *et al.* (2004) reported higher ET-1 plasma concentration in children and adolescents affected with IDDM compared to healthy subjects. Similar results have also been recorded previously among adults affected with IDDM (Sarman *et al.*, 2000; Ciarla *et al.*, 2001) as well as type 2 diabetes (Ak *et al.*, 2001; Bruno *et al.*, 2002; Schneider *et al.*, 2002).

Poor metabolic control of diabetes may increase incidence of long term complications possibly through increased production of ET-1 and hyperglycemia-mediated vascular inflammation. In the present study ET-1 levels were significantly higher in patients with poor metabolic control compared to those with ideal control [median (IQR) = 39.8 (7.4-44.4) Vs 5.0 (4.6-5.7) pg mL^{-1} respectively; p = 0.004). In a previous study Sarman *et al.* (2000) found that diabetic patients with vascular complications had significantly higher plasma ET-1 concentration than found in diabetic patients without complications. This indicates that the increased ET-1 level in diabetic patients is a marker of endothelial dysfunction and it plays an important role in the pathogenesis of diabetic complications.

Glycosylated hemoglobin derivative HbA1c is the result of nonenzymatic reaction between glucose and hemoglobin, its measurement is the best method for median to long-term diabetic monitoring. The Diabetes Control and Complications Trial (DCCT) has demonstrated that patients with HbA1c levels around 7% had the best outcomes relative to long-term complications and values more than 9% carry an increased risk of long term complications (William, 2005). In the present study ET-1 levels correlated positively with HbA1c levels. Thus in poorly controlled diabetes endothelial dysfunction follows with increase plasma levels of ET-1 and HbA1c consequently increasing the risk for long-term complications.

Among the studied patients, ET-1 correlated positively with disease duration and was significantly higher in patients with disease duration more than or equal to 4 years when compared to those with duration less than 4 years. This was in agreement with Ak *et al.* (2001) who found that elevated plasma ET-1 in type 2 diabetic patients correlated with long disease duration. Our results are in harmony with other studies performed on adults with type 2 diabetes mellitus and showing good correlation between ET-1 and increased disease duration and poor correlation with metabolic control (Ak *et al.*, 2001; Bruno *et al.*, 2002).

Regarding plasma NO level, in the present study it was not significantly different from control; this was in accordance to the findings of Telci *et al.* (2000). It was also found that patients with poor metabolic control had significantly higher NO levels when compared to those with ideal control. It is well known that hyperglycemia reduces endothelial NO production and bioavailability (Honing *et al.*, 1998; Laight *et al.*, 2000), so the elevated NO levels in the present study in patients with poor metabolic control might be a compensatory mechanism to

protect against damaging ET-1. This suggestion is further supported by our finding that NO levels correlated significantly only with ET-1 levels but not with that of HbA1c. The cause of increased NO levels in these patients may be related to ET-1 itself which leads to activation of ET_B receptors on the endothelial cells thus stimulating the synthesis of NO from these cells (Verhaar *et al*., 1998).

We can conclude that in children with IDDM poor metabolic control and increased disease duration are associated with increased ET-1 production, which may be related to future diabetic complications. The elevated plasma NO levels in poorly controlled patients might be a compensatory protective response towards the increased ET-1 production. Follow up of these patients is recommended to detect their future diabetic complications in relation to the levels of ET-1 and NO.

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