The Role of Endothelin-1 in Microvascular Dysfunction in Children with Type 1 Diabetes Mellitus

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Abstract: Most of the diabetic complications such as retinopathy, nephropathy, and neuropathy, have their basis in disturbed microvascular function. Structural and functional changes in the microcirculation are present in diabetes mellitus irrespective of the organ studied, and the pathogenesis is complex. Endothelial dysfunction, characterized by an imbalance between endothelium-derived vasodilator and vasoconstrictor substances, plays an important role in the pathogenesis of diabetic microangiopathy. Increased circulating levels of endothelin-1 (ET-1), a potent vasoconstrictor peptide, has been found in patients with diabetes. Objective: 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. 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.

INTRODUCTION

According to who health organization, diabetes mellitus (DM) now affects about 220 million people worldwide (WHO, 2009), and the growth in its prevalence represents a global health crises already accounting for more than 10% of the total healthcare expenditure in many countries (Brown, 2008).

Diabetes is a metabolic disorder which is characterized by hyperglycemia and glucose intolerance due to insulin deficiency, impaired effectiveness of insulin action or both. Type 1 diabetes mellitus is caused by cellular mediated autoimmune destruction of pancreatic islet beta cells leading to loss of insulin production. It usually starts during childhood but can occur at all ages. Type 2 DM accounts for 90%-95% of all diabetes and is more common in people older than 45 who are overweight. There is strong evidence that genetics play an important role as well. However the prevalence of type 2 DM is becoming higher in children and young adults because of higher rate of obesity in the population (H.King et al., 1998) (international diabetes federation, 2006). Despite modern insulin treatment, >50% of patients with childhood-onset type 1 diabetes developed detectable diabetes complications after approximately 12 years of onset (Svendsone 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 (Gliastras et al., 2005). The healthy endothelial monolayer is optimally positioned in order to respond to physical and chemical signals, by producing a wide range of factors that regulate vascular tone, cellular adhesion, thromboresistance, smooth muscle cell proliferation, and vessel wall inflammation. The importance of endothelium was first recognized by its effect in limiting the vascular tone (Deanfield et al., 2007).

The term endothelial dysfunction refers to a condition in which the endothelium loses its physiologic properties and shifts towards a vasoconstrictor, prothrombotic, and proinflammatory state (Potenza et al., 2009).

Endothelin was first described by Hickey et al., 1985 as a peptidergic endothelium-derived constricting factor. Three different endothelin peptides are known, endothelin 1,-2 and-3. The most abundant endothelin peptide in circulation is endotheline-1 (E1) (kedzierski and yanagisawa, 2001).

Endothelin-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 (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). Endothelin exerts its effect through two different G protein coupled receptors, the endothelin type A (ETₐ) receptor and the endothelin type B (ETᵦ) receptor (Arai et al., 1990, He et al.,2007). The endothelin ETₐ receptors are expressed in vascular smooth muscle cells and mediate vasoconstriction. In healthy conditions, endothelin ETᵦ receptors are mainly located on endothelial cells and mediate vasodilatation via the release of nitric oxide (NO), prostaglandins and endothelium-derived hyperpolarizing factor (Szok et al., 2001, Nilson et al., 2008). 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 clinic and Diabetes Unit; research institute of ophthalmology and Ein Shams Hospital, Egypt. They were 13 male and 21 female. Their ages ranged from 4 to 14 year [median (IQR) = 11 (8-12) year]. 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. 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 (HbAlc<7.6%) (n = 17), patients with moderate control (HbAlc = 7.6-9%) (n = 9) and patients with poor metabolic control (HbAlc > 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. 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 umol L. HbAlc 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 Smimov 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), HbAlc 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.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>IDDM (n = 34)</th>
<th>Control (n = 17)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endothelin-1 (pg mL(^{-1}))</td>
<td>5.9 (4.9-39.2)</td>
<td>4.9 (4.4-6.1)</td>
<td>0.02</td>
</tr>
<tr>
<td>Nitric oxide (nmol L(^{-1}))</td>
<td>24.65 (21.9-30.2)</td>
<td>22 (21-26.5)</td>
<td>0.09</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Ideal control (n=17)</th>
<th>Moderate control (n = 9)</th>
<th>Poor control (n = 8)</th>
<th>P1*</th>
<th>P2*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endothelin-1 (pg mL(^{-1}))</td>
<td>5.0 (4.6-5.7)</td>
<td>24.0 (7.5-38.1)</td>
<td>39.8 (7.4-44.4)</td>
<td>0.004</td>
<td>0.001</td>
</tr>
<tr>
<td>Nitric oxide (nmol L(^{-1}))</td>
<td>22 (20.6-22.8)</td>
<td>24.5 (22.7-31.5)</td>
<td>31 (27.6-33)</td>
<td>&lt;0.001</td>
<td>0.08</td>
</tr>
</tbody>
</table>

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 HbAlc r=0.39 p=0.02.
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.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Duration &gt; 4 years (n = 20)</th>
<th>Duration ≤4 years (n = 14)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endothelin-1 (pg mL⁻¹)</td>
<td>25.8 (12.3-38.1)</td>
<td>16.4 (5.6-20.2)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Nitric oxide (mmol L⁻¹)</td>
<td>23.7 (22.1-24.6)</td>
<td>25.9 (21.1-29.2)</td>
<td>0.066</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Nitric oxide</th>
<th>r*</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbAlc</td>
<td>0.41</td>
<td>0.10</td>
</tr>
<tr>
<td>Disease duration</td>
<td>0.33</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Discussion:

Endothelial dysfunction, characterized by an imbalance between endothelium-derived vasodilator and vasoconstrictor substances, plays an important role in the pathogenesis of vascular complications in diabetes, including microangiopathy (Creager et al 2003; Luscher et al 2003).

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). Also Kani (2008) stated that, the production of the plasma levels of ET-1 are elevated in patients with type 2 diabetes, and a positive correlation between plasma ET-1 levels and diabetic microangiopathy has been reported, suggesting a potential role of the endothelin system in the pathophysiology of vascular complications in diabetes..

Similarly (Bruno et al., 2002; Schieider et al., 2002 & Skar, 2010) reported higher ET-1 plasma concentration in patients with type 2 diabetes.

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 previous studies Sarman et al., (2000) and Yang et al., (2010), Ergul (2011), 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 HbAlc 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 HbAlc 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 HbAlc levels. Thus in poorly controlled diabetes endothelial dysfunction follows with increase plasma level of ET-1 and HbAlc 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 (Laight et al., 2000; Potenza et al., 2009) 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 HbAlc. 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.

**REFERENCES**


