BRIEF REPORT

Effect of Insulin on Plasma Vascular Endothelial Growth Factor in Children with New-Onset Diabetes

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Context: Considerable experimental evidence inculpates vascular endothelial growth factor (VEGF) as one of the candidate factors providing a mechanistic link between hyperglycemia and diabetic complications.

Aim: The aim of the study was to assess the effect of insulin treatment and glycemic control on plasma VEGF levels in children with new-onset diabetes.

Methods: This prospective study assessed the changes in plasma VEGF levels after treatment of diabetes with insulin. We also aimed to ascertain whether there was any correlation between plasma VEGF levels and simultaneous random plasma glucose. The study comprised 19 children with new-onset diabetes mellitus between the ages of 3 and 18 yr. The control group comprised 55 healthy nondiabetic children with idiopathic short stature.

Results: Plasma VEGF concentrations were significantly elevated in children at diagnosis of diabetes, compared with healthy controls (P < 0.0002). Plasma VEGF levels (P < 0.01) and hemoglobin A1C (P < 0.0001) declined in diabetic children after insulin treatment. There was a highly significant correlation between reduction in plasma VEGF levels and hemoglobin A1C levels (r = 0.65, P = 0.0037). We did not find any correlation between the simultaneous plasma glucose values and basal VEGF.

Conclusions: Presence of hyperglycemia and/or insulin deficiency in children with new-onset of diabetes is associated with plasma VEGF elevation, even at the outset of disease, and this can be mitigated by insulin therapy. (J Clin Endocrinol Metab 90: 4920–4923, 2005)

VASCULAR ENDOTHELIAL GROWTH factor (VEGF) is a highly conserved homodimeric glycoprotein, a potent cytokine, and robust vascular permeability factor (1,2). Among the five isoforms of VEGF, VEGF165 is the major isoform, and the properties of native VEGF correspond to those of VEGF165 (3). VEGF has been identified as an endothelial cell-specific mitogen and angiogenic factor that plays a cardinal role in physiologic and pathologic vasculogenesis (4,5). In diabetes, VEGF is considered to be an important mediator in the pathogenesis of endothelial dysfunction and serves a central role in mediating diabetic vasculopathy. VEGF stimulates and promotes vascular permeability, endothelium-dependent vasodilatation (6) and stimulates monocyte chemotaxis and tissue factor production, all of which contribute to micro vascular complications.

In experimental in vivo models of diabetes, VEGF has been shown to be instrumental in the development of diabetic renal changes (7,8). An inappropriate rise in VEGF production augments glomerular vascular permeability and exacerbates proteinuria (9). As further evidence of the role of VEGF, inhibition of VEGF action by administration of antibody against VEGF was found to prevent both glomerular hyperfiltration, glomerular hypertrophy, and ameliorates albuminuria (7,8,10,11). Furthermore, increases in expression of both VEGF and its receptor have been reported in the kidney in rodents with type 1 or type 2 diabetes (8,11). In humans, these finds in rodents have been corroborated: renal cortical VEGF expression is increased in the early stages of type 1 and type 2 diabetes, coinciding with renal hypertrophy, inferring that VEGF may be involved in stimulating protein synthesis (12).

Both hyperpermeability and breakdown of the blood-retinal barrier are major early functional disorders observed in diabetic retinopathy. VEGF is thought to play an essential role in intraocular neovascularization in the breakdown of the blood-retinal barrier (13).

Of the many factors that modulate VEGF expression, oxygen tension has been shown to be preeminent, both in vitro and in vivo (14). Additionally, mechanical stretch was found to induce VEGF expression in vitro in human mesangial cells and vascular smooth muscle cells (15). There is an increased production of VEGF in the setting of diabetes in humans and animal models (16,17), and hyperglycemia results in a dramatic up-regulation of VEGF expression in a variety of cell types (18,19). Furthermore, VEGF expression has also been found to be increased with vasoactive hormones (vasopresin and angiotensin) (11) and advanced glycation end prod-
ucts, growth factors like fibroblast growth factor, TGFβ1, and platelet-derived growth factor (20). Insulin was shown to increase VEGF protein expression in cultured vascular smooth muscle cells derived from human aortas and aortas of insulin-sensitive Zucker rats but not in insulin-resistant Zucker rats (21). Paradoxically, insulin and dextrose infusion together was found to suppress VEGF levels in obese, non-diabetic adults (22).

In this study, we examined the effects of insulin treatment and glycemic control on plasma VEGF levels in children newly diagnosed with diabetes. Insofar as insulin treatment induces manifold signaling cascades and resultant metabolic changes, several variables were assessed in addition to plasma VEGF. These variables included initial hemoglobin A1c (HbA1c); systemic acidosis; and a surrogate marker of dehydration, blood urea nitrogen (BUN).

Patients and Methods

This longitudinal study assessed the changes in plasma VEGF levels after treatment of diabetes with insulin over a 2-yr enrollment period. Between 2001 and 2003, all children between 3 and 18 yr of age with new-onset diabetes diagnosed at Children’s Hospital (Birmingham, AL) were asked to participate. Random plasma VEGF samples were obtained in a control group of 55 healthy nondiabetic children with idiopathic short stature as defined by a peak GH response of more than 10 mU/liter, after two provocative agents (arginine, t-dopa, or clonidine). By definition, this control group did not have any concomitant hormonal abnormalities or chronic illnesses. Subjects were enrolled after written informed consent. Ethical approval for this study was granted by the University of Alabama Institutional Review Board Ethical Committee.

The diagnosis of diabetes was established by the presence of classic symptoms, persistently elevated blood sugar, and elevated HbA1c. Using conventional criteria such as a positive glutamic acid decarboxylase and islet cell antibodies, along with classic clinical features (polyuria, polydipsia, and weight loss) were classified as type 1 diabetes. Hyperinsulinemic obese patients with negative diabetes-related antibodies were classified as type 2 diabetes. The following data were collected: demographic information (age, sex), weight, height, body mass index (BMI), HbA1c at diagnosis, serum total CO2 (tCO2), and classification of diabetes mellitus. Before commencing insulin replacement, blood was drawn for the measurement of insulin, HbA1c, tCO2, and VEGF. The dose of insulin was adjusted to target blood sugars in the range of 80–180 mg/dl. Patients who had associated hormonal abnormalities were excluded. At the initial follow-up visit, plasma VEGF levels were drawn, generally around 3–5 months after diagnosis. Likewise at this visit, changes in HbA1c, and BMI and VEGF levels were assessed. Plasma VEGF samples were stored at −80°C.

**Assays**

Plasma VEGF was assayed with the use of ELISA kits (human VEGF quantikine ELISA kit; R&D Systems, Minneapolis, MN), according to the manufacturer’s instructions. The intra- and interassay coefficients of variations were 5 and 7%, respectively. The sensitivity of the human VEGF isoform, VEGF-165, was determined using standard curves. BUN was measured by using urease quinolinium dye method, and the total CO2 was measured by enzymatic method, both obtained through the hospital clinical laboratory.

**Statistical analysis**

All statistical analyses were performed using SAS version 9.00 (SAS Institute Inc., Cary, NC). Changes in plasma VEGF and HbA1c on treatment with insulin was assessed using paired t test. Correlations between plasma VEGF and five variables [age, BMI, CO2, HbA1c, and type of diabetes mellitus (DM)] were computed by Spearman’s correlation. Statistical significance was inferred when P < 0.05. χ² tests were used to determine whether the two groups differed in gender, race, or type of DM.

**Results**

Nineteen patients with DM were enrolled in the study. Boy to girl ratio was 9:10, with mean age of 9.61 ± 0.89 yr and a mean BMI of 23.40 ± 1.61 kg/m². Three had type 2 DM, and the remaining 16 had type 1 diabetes (Table 1).

Plasma VEGF declined from a mean baseline level of 215.4 ± 37.47 to 94.19 ± 12.92 pg/ml in children with diabetes on response to insulin replacement (P < 0.0002; Fig. 1). HbA1c levels similarly decreased in response to insulin replacement (P < 0.001). Correlation between plasma VEGF change and change in HbA1c was significant (Spearman correlation).

**TABLE 1. Clinical and laboratory characteristics of patients with new-onset diabetes before and after treatment with insulin**

<table>
<thead>
<tr>
<th>Gender</th>
<th>Age (yr)</th>
<th>DM type</th>
<th>BMI (kg/m²)</th>
<th>tCO2 (mmol/liter)</th>
<th>BUN</th>
<th>VEGF before Rx (pg/ml)</th>
<th>VEGF after Rx (pg/ml)</th>
<th>VEGF change (after-before)</th>
<th>HbA1c before Rx</th>
<th>HbA1c after Rx</th>
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±SEM ± 0.89 ± 1.61 ± 1.38 ± 1.21 ± 37.47 ± 12.92 ± 41.72 ± 0.47 ± 0.35 ± 0.50

Data are expressed as mean ± SEM. Rx, Treatment.
Initial mean VEGF in diabetic patients was elevated, \( P = 0.18 \), stature) had mean plasma VEGF levels of 85.39 pg/mL. Corresponding random plasma glucose levels was detected (\( r = 0.44 \)). No correlation between initial plasma VEGF levels and age, type of diabetes, and sex or BMI. Similarly, no significant correlation between basal plasma VEGF levels and the corresponding random plasma glucose levels was detected (\( r = 0.18 \), \( P = 0.44 \)).

The controls (55 healthy children with idiopathic short stature) had mean plasma VEGF levels of 85.39 ± 10.55 pg/mL. Initial mean VEGF in diabetic patients was elevated, compared with this control group (\( P = 0.0002 \)).

**Discussion**

Considerable basic and clinical research has focused on the pathogenesis of endothelial dysfunction in diabetes, but the exact underlying mechanism remains incompletely defined. Vascular complications of chronic hyperglycemia are responsible for most of diabetes-associated morbidity and mortality. One of the principal consequences of chronic hyperglycemia is damage to vascular endothelial cells. Ischemia is the late consequence of vascular damage in patients with diabetes and this may initiate an angiogenic response. VEGF stimulates microvascular permeability, endothelium-dependent vasodilatation, and angiogenesis, and, as aforesaid, its synthesis is enhanced by hyperglycemia, advanced glycation end products, tissue hypoxia, and hypertension.

The primary factors that regulate the plasma level of VEGF in diabetes are contestable. High glucose concentration was found to induce VEGF mRNA expression and protein production in vascular smooth muscle cells (19, 23). This finding was also corroborated by animal models in which vascular dysfunction induced by elevated blood glucose levels was found to be mediated by VEGF (10). A significant correlation between plasma VEGF and both HbA1C and fasting plasma glucose in poorly controlled diabetics was found by Kakizawa et al. (24). Our subjects were different from that study population, given that children in our study were newly diagnosed children with diabetes. The study by Hovind et al. (9) suggested gender may be a factor in regulating serum VEGF levels. We did not find a correlation with gender.

Our study shows up-regulation of VEGF by hyperglycemia as evidenced by the significant increase of plasma VEGF, compared with healthy controls. Our data along with others (9, 23, 25, 26) suggest that plasma VEGF levels are disturbed with hyperglycemia, insulin deficiency, or both. On the whole, the effect of insulin replacement therapy on both HbA1C and plasma VEGF is noteworthy. Whether circulating VEGF could be a surrogate marker for tissue paracrine VEGF is presently unknown. There are suggestive data that circulating VEGF may be damaging and, moreover, correlates with the risk and degree of albuminuria (9, 23, 26).

Chiarelli et al. (23) found that serum VEGF levels significantly increased in pre pubertal and pubertal children with diabetes, compared with controls. This study design is dissimilar to ours insofar as the subjects comprised diabetic children 2 yr after diagnosis. Our patients are new-onset diabetics and were observed only for 5 months after diagnosis. We did not observe that pubertal effect in our study subjects.

Our data suggest in the presence of insulin deficiency and/or hyperglycemia in children with new-onset diabetes, there is an elevation of circulating VEGF, which can be mitigated by insulin replacement therapy. However, it was surprising to find lack of correlation between random plasma glucose and VEGF elevation in our study. This implies that chronic hyperglycemia is the culprit in diabetic complications, more so than acute hyperglycemia, and corroborates the definite role for intensive insulin management. This may be therapeutically and pathologically germane because studies (23) have shown that serum levels of VEGF represent an early marker of a generalized vascular dysfunction, and this peptide irrefutably contributes to endothelial damage in diabetes.

In conclusion, plasma VEGF levels are increased at the onset of diabetes in children before insulin therapy and improvement in glycemic control significantly reduces the elevations of plasma VEGF.

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**References**


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