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Increased Vascular Endothelial Growth Factor Serum Concentrations May Help to Identify Patients with Onset of Type 1 Diabetes during Childhood at Risk for Developing Persistent Microalbuminuria

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This study was designed to evaluate whether vascular endothelial growth factor serum concentrations may identify adolescents with onset of type 1 diabetes during childhood at greater risk to develop persistent microalbuminuria and incipient diabetic nephropathy. In January 1989, vascular endothelial growth factor serum levels were measured in 101 normoalbuminuric diabetic children and adolescents (aged 7–14.9 yr; onset of diabetes before age 18 yr; duration of diabetes >7 yr). Participants were clinically examined at baseline and annually thereafter. Vascular endothelial growth factor serum concentrations were measured every year during the 8-yr follow-up period. Over 8 yr, 11 of 101 patients (10.9%) developed persistent microalbuminuria; no patient developed overt nephropathy. The risk of developing microalbuminuria was higher in children with increased vascular endothelial growth factor serum levels (using 160 pg/ml as the arbitrary cut-off point; group 1) compared with those with normal vascular endothelial growth factor serum levels at the beginning of the study (group 2; 19.2 *vs.* 2.0%; $P < 0.01$; sensitivity, 90.9%; specificity, 53.3%). The odds ratio for the occurrence of microalbuminuria after adjustment for confounding variables (albumin excretion rate, sex, hemoglobin A_{1c}, mean blood pressure, cholesterol, and triglycerides) in type 1 diabetic adolescents with elevated vascular endothelial growth factor serum levels was 4.1 (95% confidence interval, 2.0–10.9).

These results suggest that vascular endothelial growth factor serum concentrations may be one of the predictors and risk factors for microalbuminuria and incipient diabetic nephropathy in adolescents and young adults with onset of diabetes during childhood. Persistently increased vascular endothelial growth factor serum levels may help to identify normotensive, normoalbuminuric patients with type 1 diabetes who are predisposed to develop persistent microalbuminuria later in life. (*J Clin Endocrinol Metab* 86: 3871–3876, 2001)

RENAL DISEASE remains the major cause of morbidity and mortality among patients with type 1 diabetes (1). The cumulative risk for developing diabetic nephropathy is about 30–50% after 40 yr of disease, but those who develop type 1 diabetes during childhood seem to have considerably higher risk (2). Even if diabetic nephropathy is rare in children and adolescents (3, 4), the early stages of this complication can be detected by the presence of microalbuminuria, defined as a urinary albumin excretion rate (AER) of 20–200 μg/min in two of three overnight collections obtained in 6 months (5). Microalbuminuria is the first clinically identifiable sign of risk of developing diabetic nephropathy and other vascular complications, and in particular, it is predictive of the occurrence of overt proteinuria and cardiovascular complications, particularly in IDDM (6).

The identification of high risk patients before the onset of microalbuminuria would have great importance in pinpointing subjects with diabetes who might benefit from a more precocious and aggressive medical treatment aimed at arresting the progression to the later stages of the disease.

Mean glycated hemoglobin (HbA_{1c}) is the dominant predictor of nephropathy, as demonstrated by cross-sectional and longitudinal studies (7, 8). Yet despite maintenance of good metabolic control, 10–15% of patients develop diabetic nephropathy after 20 yr of diabetes duration (8); this evidence suggests that additional factors may contribute to determine the risk of developing diabetic nephropathy, but, unfortunately, provides no explanation for the mechanisms responsible (9).

The structural changes occurring in early diabetic nephropathy, characterized by renal hypertrophy and increased microvascular permeability, suggest a causal role for impairment of the growth factor network (10). A large amount of evidence supports the hypothesis that a panel of growth factors, in particular the GH/IGF-I axis and TGFβ isoforms, are involved in the development of diabetic nephropathy through a complex intrarenal system (10). Together with them vascular endothelial growth factor (VEGF) is one of the most likely candidates for the development of both retinopathy and nephropathy, even if evidence for the latter is less extensive to date (11, 12). VEGF is a cytokine with potent vascular permeability and angiogenic effects and has been implicated in the pathogenesis of vascular-related diseases, such as growth of tumors, atherosclerosis of coronary arteries in ischemic heart disease, Kawasaki disease, neovascularization in synovial tissue in rheumatoid arthritis, and diabetic microangiopathy (13, 14).

VEGF expression and binding have been detected in the

Abbreviations: AER, Albumin excretion rate; AGE, advanced glycation end product; HbA_{1c}, glycated hemoglobin; OR, odds ratio; VEGF, vascular endothelial growth factor.

kidney of rats (12) and humans, in particular in the epithelial cells of the glomerulus, podocyte, and collecting ducts (15). The same stimuli have been shown to increase the expression of VEGF as well as of the other growth factors implicated in the development of diabetic nephropathy: hypoxia, vasopressor hormones such as vasopressin and angiotensin II, advanced glycation end products (AGEs), mechanical strain, cytokines such as TGFβ, and growth factors such as fibroblast growth factor and platelet-derived growth factor (10). Furthermore, TGFβ and IGF-I have been reported to enhance VEGF production in fibroblastoid and epithelial cells and in retinal cells, respectively (10). These links between VEGF and other growth factors whose roles in the pathogenesis of diabetic nephropathy are well established suggest that VEGF may act in concert with them as a causal factor and eventually may represent a marker for the capacity of an individual to produce a potentially detrimental molecule in the development of diabetic complications.

Increased serum concentrations of immunoreactive VEGF have been described in adults with type 2 diabetes and diabetic nephropathy (16). Data from our group have shown that serum VEGF concentrations are increased in prepubertal and pubertal children with type 1 diabetes, and that there is a direct correlation between serum levels of VEGF and the severity of complications (17).

To the best of our knowledge, no long-term study has been performed in children and adolescents with type 1 diabetes mellitus. In the present study plasma VEGF levels were evaluated in a large group of adolescents with onset of diabetes during childhood to determine whether increasing VEGF levels may predict the occurrence of persistent microalbuminuria.

Subjects and Methods

Patients

The study started in January 1989. The patients were selected from those regularly attending the Department of Medicine and the Department of Pediatrics, University of Chieti (Chieti, Italy). To be eligible for participation, the patients had to fulfill the following inclusion criteria: onset of diabetes before the age of 18 yr, duration of disease longer than 7 yr, insulin treatment from the time of diagnosis, and normal blood pressure.

One hundred and one patients (52 females and 49 males, aged 7–14.9 yr) satisfied the inclusion criteria and agreed to participate. They were arbitrarily divided into 2 groups on the basis of VEGF serum concentrations: group 1 had levels above and group 2 had levels below 160 pg/ml. The sensitivity positive, the predicting value for the occurrence rate of microalbuminuria, and the specificity negative, the predicting value for the occurrence rate of microalbuminuria, were calculated. One hundred and two healthy subjects, matched for age and sex, were also studied as control groups. The two groups of patients with type 1 diabetes were comparable for age, duration of diabetes, body mass index and biochemical parameters, HbA_{1c}, AER value, and systolic and diastolic blood pressures. The baseline clinical characteristics of the 2 groups of type 1 diabetic patients who completed the study are summarized in Table 1.

During the study, patients did not undergo antihypertensive therapy and had no clinical or biochemical evidence of renal disease. At the beginning of the study, all patients showed an AER less than 20 μg/min in three overnight urine collections obtained over 6 months and repeated during the next 6 months.

Patients and their families gave informed consent to participate, and the study was approved by the ethics committee of University of Chieti.

Quantification of VEGF concentrations in serum

VEGF serum levels were measured in all samples in duplicate by a sensitive and highly specific colorimetric ELISA with slight modifications of a chemiluminescence enzyme immunoassay method previously described (18). The recombinant human VEGF121 was purified from the culture medium of the transformed yeast (19). The antihuman VEGF polyclonal antibody was prepared from rabbit serum immunized with the recombinant human VEGF121-glutathione-*S*-transferase fusion protein (20). The specificity of this anti-VEGF polyclonal antibody was well demonstrated by other investigators (18–20). As the cross-reactivity between this assay and VEGF165 (which is the most abundant VEGF isoform in the blood) is 92%, we can assess that plasma levels of VEGF121 reliably represent concentrations in serum. The minimal detectable VEGF concentration was 8.0 pg/ml. There were 16 values of VEGF under the detection limit; undetectable values were assigned the detection limit. Intra- and interassay coefficients of variation were 5.1% and 6.2%, respectively. To assess the consistency of VEGF determination over the 9-yr follow-up, we measured serum VEGF concentrations in the same samples after 9 yr, and we achieved similar results (interassay variation, 7.4%).

Other analyses

A number of parameters were determined in all patients at baseline and every 6 months: HbA_{1c}, plasma electrolytes, cholesterol, triglycerides, serum creatinine, AER from three 24-h urine collections, body weight, and arterial blood pressure.

HbA_{1c} was measured throughout the 8-yr study by HPLC (Bio-Rad Laboratories, Inc., Richmond, CA); the mean of at least four HbA_{1c} determinations in 1 yr was considered the index for glycemic control. The mean $\pm$ SD HbA_{1c} value in the control population was 4.8 $\pm$ 0.1%. Our HbA_{1c} method has been compared with an international reference laboratory at Steno Memorial Hospital in Copenhagen; the correlation between the two laboratories was significant ($y = 0.9703x + 0.387$; $r^2 = 0.9709$; $P < 0.001$), as previously described (21). The creatinine concen-

TABLE 1. Baseline clinical features of type 1 diabetic children and adolescents who completed the study

	Group 1	Group 2	*P* value
n	52	49	
Sex (F/M)	27/25	25/24	NS
Age (yr)	14.2 $\pm$ 3.9	14.7 $\pm$ 4.0	NS
Duration of diabetes (yr)	12.3 $\pm$ 3.4	12.6 $\pm$ 3.5	NS
Blood pressure (mm Hg)			
Systolic	112 $\pm$ 7	110 $\pm$ 8	NS
Diastolic	76 $\pm$ 6	75 $\pm$ 7	NS
HbA_{1c} (%)	8.1 $\pm$ 2.1	8.0 $\pm$ 2.3	NS
Cholesterol (mmol/liter)	3.8 $\pm$ 1.5	4.0 $\pm$ 1.6	NS
Triglycerides (mmol/liter)	1.04 $\pm$ 0.98	1.05 $\pm$ 0.94	NS
Plasma creatinine (μmol/liter)	68 $\pm$ 17	70 $\pm$ 18	NS
Creatinine clearance (ml/min $1.73^{-1}\cdot m^{-2}$)	114 $\pm$ 62	111 $\pm$ 67	NS
Insulin requirement (IU/day·kg)	0.97 $\pm$ 0.53	0.94 $\pm$ 0.60	NS

Data are the mean $\pm$ SD. No patient had background retinopathy or persistent microalbuminuria.

tration was measured by an autoanalyzer (Astra 8, Beckman Instruments, Inc./Hybritech, Palo Alto, CA) (22).

AER was measured by RIA (Pharmacia, Uppsala, Sweden) as described previously (13). Persistent microalbuminuria was defined as an AER more than 20 μg/min in at least two of three overnight urine collections in 6 months. Intermittent microalbuminuria was defined as an AER not constantly more than 20 μg/min in at least three subsequent determinations in 6 months. All subjects were weighed wearing indoor clothing without shoes, and height was recorded as well. Blood pressure was measured according to the recommendations of the Task Force on Blood Pressure Control in Children (23, 24) and the American Heart Association (25). Blood was collected in the morning after an overnight fast and before the usual morning insulin injection.

Patients were advised to follow the same dietary intakes advised for the general diabetic population. A normocaloric diet was prescribed, with carbohydrate intake ranging from 54–60%, protein intake from 12–14%, salt intake less than 120 mEq/liter·73 m^{-2}/d, and cholesterol intake less than 100 mg/1000 calorie. Diet was adjusted on the basis of dietary habits of the families and children; all patients and their families received dietary education to improve dietary compliance and children's diet, according to the Italian government's recommended dietary allowances.

Statistical analysis

Values are given as the mean ± SD or as the median with the range. Comparison between the values observed during the follow-up period in groups 1 and 2 used a two-factor ANOVA with VEGF serum level category and time (baseline and follow-up period) as the factors when a significant interaction was shown by ANOVA. The χ^2 tests, two-tailed *t* test, or Mann-Whitney nonparametric test was used to assess the degree of statistical significance of differences.

As the descriptive measure of association between risk factors and each of the outcomes, odds ratios (ORs) have been given together with 95% confidence intervals. These ORs were based on the cumulative incidence rates of microalbuminuria and macroalbuminuria during the 8-yr follow-up interval (the average in the two pooled groups). Multiple logistic regression analysis was used to study the relationship between the occurrence of microalbuminuria (dependent variable) and the baseline rate of serum VEGF concentrations (independent variable) while simultaneously controlling for sex and baseline AER and the mean values of plasma creatinine, HbA_{1c}, and systolic and diastolic blood pressure levels every 6 months during the follow-up period. These independent variables were chosen because of their univariate associations with the occurrence rate of microalbuminuria. The occurrence rate of persistent microalbuminuria was treated as a dichotomy (AER >20 μg/min *vs.* AER <20 μg/min) and as a continuous numerical value [cumulative absolute change in AER (micrograms per min) from baseline]. Statistical analyses were carried out using 6.1 Base System software (SPSS, Inc., Chicago, IL).

Results

VEGF serum concentrations

The median value of VEGF serum levels in the type 1 diabetic patients who took part in the study (101 subjects) was 160 pg/ml. Therefore, the cut-off value of 160 pg/ml was used as a reasonable number to have the most acceptable number of false negative and false positive results among subjects with diabetes. In healthy control subjects, the mean ± SD serum VEGF concentration was 117.4 ± 16.3 pg/ml. Of the patients with diabetes at the beginning of the study, 52 (27 females and 25 males) had values higher than and 49 (25 females and 24 males) had values lower than the median level and were assigned to groups 1 and 2, respectively. No significant changes in serum VEGF levels were observed between baseline, 2-yr, 4-yr, 6-yr, and 8-yr measurements in each group (mean ± SD, 177.4 ± 26.9, 174.6 ± 23.9, 180.3 ± 26.8, 184.3 ± 21.2, and 186.1 ± 22.7 pg/ml in group 1; 140.1 ± 19.7, 143.4 ± 20.3, 145.6 ± 19.1, 142.1 ± 23.4, and 146.2 ± 21 pg/ml in group 2; Fig. 1).

Rate of development of microalbuminuria

During the entire follow-up period, group 1 patients had significantly higher mean HbA_{1c} values than group 2 patients (9.4 ± 1.9 *vs.* 7.9 ± 2.0; $P < 0.01$; Fig. 2). Systolic and diastolic blood pressure levels were slightly, but significantly, higher in group 1 than in group 2, although they were within the normal range (124 ± 12 *vs.* 118 ± 9 mm Hg; $P < 0.01$; Fig. 2). No differences were observed with regard to other clinical or biochemical parameters.

The number of patients who developed persistent microalbuminuria during the follow-up period in the overall population of adolescents with diabetes was significantly higher in group 1 than in group 2 (Table 2). No significant differences were observed with regard to the rate of development of intermittent microalbuminuria (Table 2). The risk of developing persistent microalbuminuria was higher in children with serum VEGF levels higher than 160 pg/ml (19.2% *vs.* 2.0%; sensitivity, 90.9%; specificity, 53.3%). The specificity was better (85.1%) if the cut-off was fixed at a VEGF concentration of 180 pg/ml, but with this cut-off value the sensitivity was much lower (76.2%; Table 3). Therefore, the VEGF cut-off value of 160 pg/ml has the most acceptable number of false negative and false positive results among adolescents and young adults with diabetes (Table 3). The OR for the occurrence of microalbuminuria after adjustment for the mentioned confounding variables, such as AER, sex, HbA_{1c}, mean blood pressure, cholesterol, and triglycerides, in type 1 diabetic patients with VEGF values higher than 160 pg/ml was 4.1 (95% confidence interval, 2.0–10.9). Similar results were observed using the cumulative absolute increase in AER above baseline as the continuous numerical variable.

Discussion

The present study shows that patients with increased VEGF serum concentrations have an increased risk to develop persistent microalbuminuria. These results are in agreement and extend previous findings from our group showing that serum levels of VEGF are increased in preschool, prepubertal, and particularly pubertal patients with

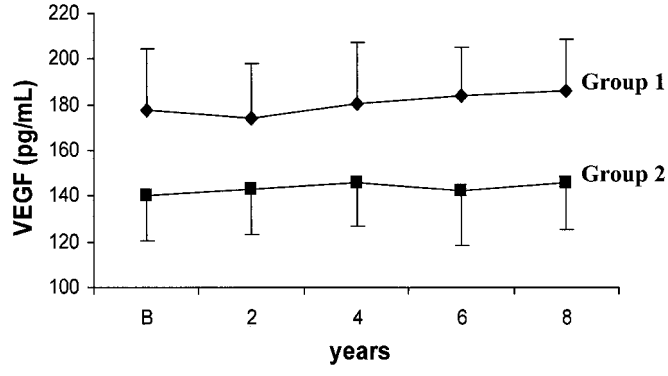

FIG. 1. VEGF serum concentrations in diabetic children and adolescents at baseline (B) and at the 2-, 4-, 6-, and 8-yr follow-ups. Type 1 diabetic patients were divided into group 1 (♦; those with VEGF >160 pg/ml) and group 2 (■; those with VEGF <160 pg/ml).

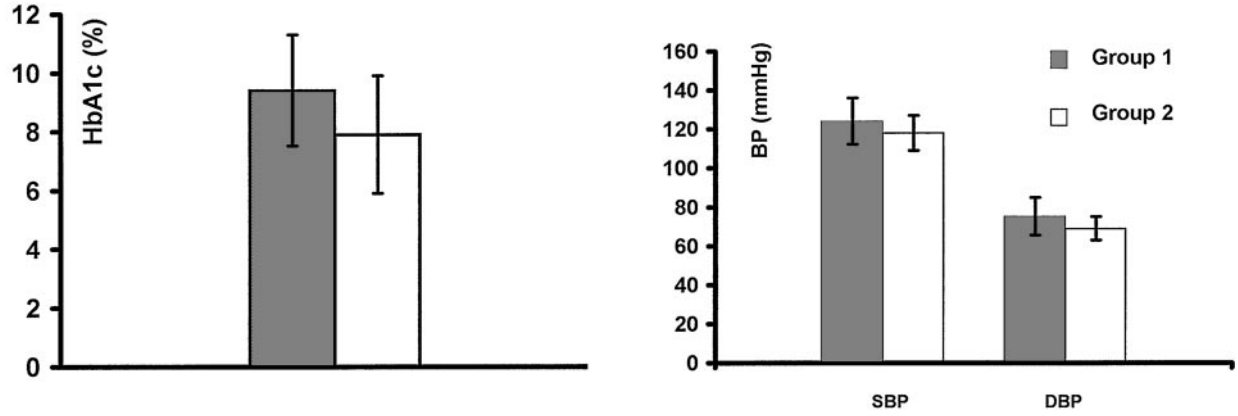

FIG. 2. Mean HbA_{1c} and blood pressure (SBP, systolic blood pressure; DBP, diastolic blood pressure) during the 8-yr period of follow-up in patients with VEGF above 160 pg/ml and those with VEGF below 160 pg/ml.

TABLE 2. Development of persistent or intermittent microalbuminuria in group 1 and group 2

	Group 1	Group 2	*P* value
Duration of follow-up (yr)	8.3 (8.1–8.9)	8.4 (8.1–9.0)	NS
Persistent microalbuminuria	10/52	1/49	<0.01
Intermittent microalbuminuria	4/52	3/49	NS

Data are means (range) or n/total.

TABLE 3. VEGF serum concentrations as predicting factor of the rate of occurrence of microalbuminuria in type 1 diabetic adolescents and young adults overall during the follow-up period

Cut-off value (pg/ml)	Sensitivity (%)	Specificity (%)
140	96.1	30.6
160	90.9	53.3
180	76.2	85.1
200	52.6	93.8

diabetes compared with controls, and that this increase is related to the severity of nephropathy and retinopathy in adolescents and young adults with onset of diabetes during childhood (17). These reports give relevance to the hypothesis that VEGF could play a pivotal role in the development and progression of vascular complications in diabetes.

In addition to the well established role of this cytokine in both nonproliferative and proliferative retinopathy (10), a large body of evidence *in vitro* and in animal models suggests that this pathological effector is also induced in other tissues, namely the kidney, by the same or different stimuli. A very recent study indicates an early and persistent increase in renal VEGF gene expression in association with experimental diabetes as well as an early and transient increase in renal VEGF receptors in streptozotocin-induced diabetic rats (12). Because the main biological effect of this growth factor consists in promoting angiogenesis and permeability (14), its overexpression in the kidney might represent an excessive compensatory mechanism in response to renal injury, leading to increased albumin permeability and the occurrence of albuminuria.

It is likely that VEGF together with other growth factors could act as a distal effector of hyperglycemia-accelerated pathways and metabolic perturbations occurring in diabetes. These include increased polyol pathway activity and associated changes in intracellular redox state (13), increased diacylglycerol synthesis with consequent activation of specific PKC isoforms, increased nonenzymatic glycation of both intracellular and extracellular proteins, elevated angiotensin II, hypoxia, increased formation of reactive oxygen species, and mechanical stretch (10). The up-regulation of VEGF expression triggered by the aforementioned stimuli in the kidney provides an explanation and suggests a plausible source for the increased VEGF levels detected in the serum of our patients.

VEGF serum levels are likely to be continuous; the selection of VEGF cut-off of 160 pg/ml is arbitrary and was made in response to the analyses to achieve the most acceptable sensitivity and specificity.

We also found that glycemic control was poorer in patients who more frequently developed persistent microalbuminuria later in life (group 1); in fact, the mean HbA_{1c} values during the entire follow-up were significantly higher in group 1 than in group 2. Chronic hyperglycemia is able to increase the formation and accumulation of AGE in the kidneys of diabetic rats and in the serum of children and adolescents with type 1 diabetes (26); this overexpression of AGE, in turn, may initiate a positive feedback loop for increasing VEGF levels, as improvement of long-term glycemic control is able to reduce VEGF levels in preschool, prepubertal, and pubertal patients. In this perspective, glycemic control may be considered the major determinant of VEGF overexpression in diabetes.

On the other hand, in our study, despite an increase in HbA_{1c} as well as in blood pressure during the follow-up period, no significant changes in serum VEGF levels were observed between baseline, 2-yr, 4-yr, 6-yr, and 8-yr measurements. Therefore, it is unlikely that increased HbA_{1c} or blood pressure in group 1 was able to affect the trend of serum VEGF concentrations, at least for the duration of the observed 8-yr period of follow-up. In accordance with this observation, recent data support the hypothesis of a genetic basis in the regulation of growth factor expression: thanks to the study of TGFβ polymorphism, it was demonstrated that the TGFβ circulating concentration is predominantly under genetic control, and very recent data show gene polymorphism of VEGF to be correlated with VEGF protein production (27, 28).These findings might relieve environmental factors of the responsibility of growth factor up-regulation,

leaving them the role of enhancers, accelerating a process that is already genetically determined.

The persistently elevated VEGF concentrations detected in our study, several years before the onset of persistent microalbuminuria, suggest that expression of VEGF may be at least in part genetically determined and, as such, a valid marker for future development of complications; therefore, further studies are needed on VEGF gene polymorphism in patients with diabetes.

In patients with diabetes, microalbuminuria is a predictor of widespread severe microangiopathy and macroangiopathy. Patients with microalbuminuria show generalized dysfunction of the vascular endothelium, which may underlie the propensity of microalbuminuric patients to develop severe extrarenal vascular disease. Endothelial dysfunction has been shown to be important in the development of microalbuminuria (29, 30). Thus, in cross-sectional studies in patients with type 1 diabetes mellitus and microalbuminuria, the vascular endothelium tends to increase, rather than decrease, vascular resistance (31); fails to restrict the passage of macromolecules (1); and loses its anticoagulant and profibrinolitic properties (30, 32). In addition, there is an increase in the plasma concentrations of markers of endothelial injury and dysfunction, such as von Willebrand factor, a glycoprotein involved in primary hemostasis and secreted mainly by endothelial cells (30, 33); more importantly, endothelial dysfunction, as estimated by plasma von Willebrand factor concentration, precedes the development of microalbuminuria in insulin-dependent diabetic patients by as much as 3 yr (34).

Because microalbuminuria reflects a more generalized vascular process that affects the glomeruli, the retina, and the intima of large vessels simultaneously (1, 35), it is possible to hypothesize that VEGF may represent a reliable and early marker of this generalized vascular dysfunction and may contribute to endothelial damage in young patients with diabetes.

In conclusion, our data suggest, on the one hand, that longitudinal monitoring of a panel of plasma markers such as that used in the current study may better define their relevance in progressive kidney disease and provide greater insight into the mechanisms underlying these processes. On the other hand, a noninvasive estimation of VEGF concentrations may be a reliable method of predicting renal involvement. As microalbuminuria occurs relatively late in the disease process, evaluation of serum concentrations of VEGF may become a useful prognostic test in the earliest stages of the disease and could help to identify children and adolescents at higher risk of developing nephropathy later in life, who might benefit from a earlier and more aggressive medical intervention.

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References

1. **Deckert T, Feldt-Rasmussen B, Borch-Johnsen K, Jensen T, Kofoed-Enevoldsen A** 1989 Albuminuria reflects widespread vascular damage: the Steno hypothesis. Diabetologia 32:219–226
2. **Anderson AR, Christiansen JS, Andersen JK, Deckert T** 1983 Diabetic nephropathy in type 1 diabetes: an epidemiological study. Diabetologia 25: 496–501
3. **Rudberg S, Dahlquist G** 1996 Determinants of progression of microalbuminuria in adolescents with IDDM. Diabetes Care 19:369–371
4. **Cook J, Daneman D** 1990 Microalbuminuria in adolescents with insulin-dependent diabetes: prevalence and associated characteristics. Am J Dis Child 144:234–237
5. **Dahlquist G, Rudberg S** 1987 The prevalence of microalbuminuria in diabetic children and adolescents and its relationship to puberty. Acta Paediatr Scand 79:795–800
6. **Mogensen CE** 1990 Prediction of clinical diabetic nephropathy in IDDM patients. Are there alternatives to microalbuminuria? Diabetes 30:761–767
7. **Krolewski AS, Laffel LMB, Krolewski M, Quinn M, Warram JH** 1995 Glycated hemoglobin and risk of microalbuminuria in patients with insulin-dependent diabetes mellitus. N Engl J Med 332:1252–1255
8. **Diabetes Control and Complications Trial Research Group** 1993 The effect of intensive treatment of diabetes on development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med 329:977–986
9. **Doria A, Warram JH, Krolewski AS** 2000 Genetic susceptibility to nephropathy in IDDM; from epidemiology to molecular genetics. Diabetes Metab Rev 11:287–314
10. **Chiarelli F, Santilli F, Mohn A** 2000 Role of growth factors in the development of diabetic complications. Horm Res 53:53–67
11. **Aiello LP, Avery R, Arrig P** 1994 Vascular endothelial growth factor in ocular fluid of patients with diabetic retinopathy and other retinal disorders. N Engl J Med 331:1480–1487
12. **Cooper ME, Vranes D, Youssef S, et al.** 1999 Increased renal expression of vascular endothelial growth factor (VEGF) and its receptor VEGFR-2 in experimental diabetes. Diabetes 48:2229–2239
13. **Tilton R, Kawamura T, Chang KC, et al.** 1997 Vascular dysfunction induced by elevated glucose levels in rats is mediated by vascular endothelial growth factor. J Clin Invest 99:2192–2202
14. **Folkman J** 1995 Angiogenesis in cancer, vascular, rheumatoid and other disease. Nat Med 1:27–31
15. **Simon M, Rockl W, Hornig C, et al.** 1998 Receptors of vascular endothelial growth factor/vascular permeability factor (VEGF/VPF) in fetal and adult human kidney: localisation and [125] VEGF binding sites. J Am Soc Nephrol 9:1032–1044
16. **Wasada T, Kawahara R, Katsumory K, Naruse M, Omori Y** 1998 Plasma concentrations of immunoreacive vascular endothelial growth factor and its relation to smoking. Metabolism 47:27–30
17. **Chiarelli F, Spagnoli A, Basciani F, et al.** 2000 Vascular endothelial growth factor (VEGF) in children, adolescents and young adults with type I diabetes mellitus: relation to glycaemic control and microvascular complications. Diabet Med 17:650–656
18. **Hatanani M, Tanaka Y, Kondo S, Ohmori L, Suzuki H** 1995 Sensitive chemiluminescence enzyme immunoassay for vascular endothelial growth factor/vascular permeability factor in human serum. Biosci Biotechnol Biochem 59: 1958–1959
19. **Kondo S, Matsumoto T, Yokoyama Y, Ohmori L, Suzuki H** 1995 The shortest isoform of vascular endothelial growth factor (VEGF/VPF) produced by Saccharomyces cerevisisae promotes both angiogenesis and vascular permeability. Biochim Biophys Acta 1243:195–202
20. **Kondo S, Asano M, Suzuki H** 1993 Significance of vascular endothelial growth factor/vascular permeability factor for solid tumor growth, and its inhibition by the antibody. Biophys Res Commun 194:1234–1241
21. **Mortensen HB, Robertson KJ, Aanstoot H-J, et al.** 1998 for the Hvidøre Study Group on Childhood Diabetes. Insulin management and metabolic control of type I diabetes mellitus in childhood and adolescence in 18 countries. Diabet Med 15:752–759
22. **Allen TJ, Cooper ME, Gilbert RE, Winikoff J, Skinner SL, Jerums G** 1996 Serum total renin is increased before microalbuminuria in diabetes. Kidney Int 50:902–907
23. **Chiarelli F, Verrotti A, Morgese G** 1995 Glomerular hyperfiltration increases the risk of developing microalbuminuria in diabetic children. Pediatr Nephrol 9:154–158

24. **Task Force on Blood Pressure Control in Children** 1987 Report of the Second Task Force on Blood Pressure Control in Children. Pediatrics 79:1–26
25. **Kirkendall WM, Feinleib M, Freis ED, Mark AL** 1981 Recommendation for human blood pressure determinations by sphygmomanometers. Hypertension 3:510–519
26. **Chiarelli F, de Martino M, Mezzetti A, et al.** 1999 Advanced glycation end products in children and adolescents with diabetes: relation to glycemic control and early microvascular complications. J Pediatr 134:486–491
27. **Grainger DJ, Heathcote K, Chiano M, et al.** 1998 Genetic control of the circulating concentration of transforming growth factor type β1. Hum Mol Genet 8:93–97
28. **Watson CJ, Webb NJ, Bottomley MJ, Brenchley PE** 2000 Identification of polymorphisms within the vascular endothelial growth factor (VEGF) gene: correlation with variation in VEGF protein production. Cytokine 12:1232–1235
29. **Zenere BM, Arcado G, Saggiani F, Rossi L, Muggeo M, Lechi A** 1995 Noninvasive detection of functional alterations of the arterial wall in IDDM patients with and without microalbuminuria. Diabetes Care 18:975–982
30. **Jensen T, Bjerre-Knudsen J, Feldt-Rasmussen B, Deckert T** 1989 Features of endothelial dysfunction in early diabetic nephropathy. Lancet 1:461–463
31. **Elliott TG, Cockcroft JR, Groop PH, Viberti GC** 1993 Inhibition of nitric oxide synthesis in forearm vasculature of insulin-dependent diabetic patients: blunted vasoconstriction in patients with microalbuminuria. Clin Sci 83: 687–693
32. **Gruden G, Cavallo-Perin P, Bazzab M, Stella S, Vuolo A, Pagano G** 1994 PAI-1 and factor VII activity are higher in insulin-dependent diabetes mellitus patients with microalbuminuria. Diabetes 43:426–429
33. **Stehouwer CDA, Stroes ESG, Hackeng WHL, Mulder PGH, Den Ottolander GJH** 1991 von Willebrand factor and development of diabetic nephropathy in insulin-dependent diabetes mellitus. Diabetes 40:971–976
34. **Stehouwer CDA, Fischer HRA, van Kuijk AWR, Polak BCP, Donker AJM** 1995 Endothelial dysfunction precedes development of microalbuminuria in IDDM. Diabetes 44:561–564
35. **Messent JWC, Elliott TG, Hill RD, Jarrett RJ, Keen H, Viberti GC** 1992 Prognostic significance of microalbuminuria in insulin-dependent diabetes mellitus: a twenty-three year follow-up study. Kidney Int 41:836–839