Correlation Between Carotid Artery Distensibility and Serum Vascular Endothelial Growth Factor Concentrations in Type 1 Diabetic Subjects and Nondiabetic Subjects

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The relationships between serum vascular endothelial growth factor (VEGF) concentrations and vessel wall ultrasonic characteristics in type 1 diabetic and nondiabetic subjects were assessed. Serum VEGF concentration was measured, and ultrasound imaging and blood pressure recordings were performed in 41 type 1 diabetic subjects (hemoglobin A_{1c} [HbA_{1c}], 7.63 [1.17%]; duration of diabetes, 12 (0 to 23) years), and 50 nondiabetic subjects. Change in carotid artery luminal diameter during the cardiac cycle was measured using M-mode ultrasound, from which percentage increase in carotid artery luminal diameter was calculated; the carotid artery distensibility index was calculated as the ratio of percentage increase in carotid artery luminal diameter and pulse pressure. Serum VEGF concentration was higher in the diabetic subjects (217 [135 to 336] *v* 137 [80 to 237] pg/mL; *P* = .009). The percentage increase in carotid luminal diameter during the cardiac cycle was not significantly different between the 2 groups (12.9 [10.2 to 15.7] *v* 13.0 [10.6 to 15.0%]; *P* = .270) despite significantly greater pulse pressure in the type 1 diabetic group (55 [45 to 71] *v* 46 [41 to 51] mm Hg; *P* = .0003). The distensibility index was therefore lower in the diabetic subjects (0.24 [0.10] *v* 0.28 [0.08%]/mm Hg; *P* = .031). There was a significant negative correlation between serum VEGF concentrations and mean percentage increase in carotid luminal diameter during the cardiac cycle in the diabetic group (*r* = -.36, *P* = .021) and in the nondiabetic group (*r* = -.28, *P* = .047). This negative correlation could be strengthened by relating mean percentage increase in luminal diameter to pulse pressure to give the distensibility index. Therefore, serum VEGF concentrations correlated strongly and inversely with the distensibility index in the diabetic group (*r* = -.49, *P* = .001), in the nondiabetic group (*r* = -.29, *P* = .041), and in both groups analyzed together (*r* = -.42, *P* < .0001). Vessel wall distensibility may be an important determinant of serum VEGF concentrations in both diabetic and nondiabetic populations and may underlie the previously observed association between blood pressure and serum VEGF concentrations. The pathophysiologic relevance of these findings remains to be elucidated.
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MANY STUDIES HAVE suggested a role for vascular endothelial growth factor (VEGF) in the pathogenesis of diabetic microvascular and macrovascular disease. Perhaps the strongest case can be made for VEGF as the growth factor responsible for new vessel formation in proliferative diabetic retinopathy, with the observations that levels are increased in the vitreous of eyes from diabetic subjects with proliferation.[1,2] Furthermore, proliferation in an ischaemic mouse model can be blocked by inhibitors of VEGF.[3,4] The role of VEGF in the etiology of large vessel disease is more speculative. Extensive expression of VEGF has been demonstrated in arteries narrowed by atherosclerotic plaque,[5] in which it has been proposed that the role of VEGF may involve maintenance and repair of luminal endothelium, but it may also increase vessel permeability, allowing access of inflammatory cells and atherogenic lipoproteins to the subendothelial space.

VEGF is readily measured in both serum and plasma, with considerably lower levels found in the latter. It is not presently known whether measured levels of circulating VEGF are of biologic relevance, and the present work is a further step towards answering that question. Our previous studies suggested that blood pressure was the strongest correlate of serum VEGF concentrations in both diabetic and nondiabetic subjects,[6] and if this observation were to be confirmed, it would open up new avenues of research into the relationship between hypertension and vascular damage. We therefore sought to extend our findings by examining the relationship between serum VEGF levels and ultrasonic measurements of vessel wall characteristics. By directly examining the vessel wall during the cardiac cycle, we hoped to relate the findings in our previous studies to recent observations that mechanical stretch of vascular smooth muscle cells[7] and myocardium[8] increases VEGF expression. We therefore hypothesized that stretch of vascular smooth muscle cells in vivo caused by the pressure changes resulting from the cardiac cycle determines serum levels of VEGF. In this study, we have examined a group of subjects with type 1 diabetes in view of the increased risk of both vascular disease and hypertension in this group. We have also examined a group of nondiabetic subjects.

SUBJECTS AND METHODS

Subjects

Type 1 diabetic subjects were recruited consecutively from the diabetic clinics at St Mary's and St Charles Hospitals in London, UK; subjects had required insulin treatment within 1 year of diagnosis of diabetes and had undetectable fasting C-peptide concentrations (<100 pmol/L). Subjects with creatinine concentrations greater than 140 μmol/L or with other significant medical conditions were not included. Healthy nondiabetic control subjects were recruited from hospital staff. Local Ethical Committee approval and informed consent were obtained from all subjects.

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VEGF Measurements

Venous blood was taken from all subjects into plain tubes, and serum was prepared by gentle centrifugation at 1,000×*g* for 30 minutes. The serum was then frozen and stored at –70°C until assayed for VEGF. $VEGF_{165}$, the most abundant diffusible VEGF isoform in humans, was assayed using a quantitative sandwich enzyme immunoassay technique (R&D Systems Europe Ltd, Abingdon, UK). Mean intraassay coefficients of variation for serum VEGF are 4.5% at 235 pg/mL and 6.7% at 54 pg/mL; mean interassay coefficients of variation for serum VEGF are 7.0% at 250 pg/mL and 8.8% at 65 pg/mL.

Fasting blood was also taken for glucose concentrations and hemoglobin A_{1c} (HbA_{1c}). A single early morning urine sample was collected for measurement of albumin to creatinine ratio; a ratio of greater than 2.0 was taken to indicate the presence of microalbuminuria. Standard laboratory assays were used.

Ultrasonic Measurement of Vascular Parameters

Technique. All type 1 diabetic and nondiabetic control subjects attended for ultrasound scans. All examinations were performed using an ATL 3000 HDI system (Advanced Technology Laboratories, high-definition imaging, Seattle, WA). A 7-4 MHz linear array transducer with broad band technology was used to scan all subjects. Ultrasound parameters (postprocessing map, dynamic range, persistence, power output, and transmit gain control) were preset and kept constant throughout the study. Magnification and depth could be adjusted depending on patient anatomy and size.

Regular use of an RMI (model 415) (Gammex Radiation Measuring Instruments, Nottingham, UK) test object ensured system accuracy in sensitivity, distance measurements, and axial and lateral resolution measurements. A single experienced ultrasonographer performed all measurements, and images were stored on magneto-optical discs for analyses.

Luminal Diameter Measurements

Time Motion mode (TM-mode or M-mode) ultrasound was used to measure minimum and maximum carotid luminal diameters during the cardiac cycle. M-mode provides time and motion echo information derived from a stationary ultrasound beam placed on B-mode image (the M-line). The M-line was positioned 2.0 cm proximal to the carotid bifurcation. Two minimum and 2 maximum diameters were measured at the same point of the cardiac cycle, using electrocardiographic monitoring synchronized with ultrasound M-mode traces; the mean of the 2 values for both minimum and maximum diameters was calculated. A cross-section through the carotid artery can be considered to represent a ring of vascular smooth muscle cells. The percentage increase in carotid luminal circumference, mathematically the same as percentage increase in diameter, therefore represents the percentage increase in length of the ring of vascular smooth muscle cells, that is, the degree of stretch. Minimum and maximum carotid luminal diameters were measured in both carotid arteries, from which percentage increase in left and right carotid luminal diameters were calculated. Mean percentage increase in diameter was calculated as the mean of the left- and right-sided values.

Blood Pressure Measurements

Blood pressure was measured at the right brachial artery using a mercury sphygmomanometer. Blood pressure was taken as the mean of 2 readings taken immediately before (after resting supine for 10 minutes) and immediately after the ultrasound measurements.

Distensibility Index Measurements

The ratio of the percentage increase in carotid luminal diameter to the pulse pressure provides a measure of the distensibility of the carotid artery,[9] called the distensibility index. Therefore, mean percentage increase in carotid luminal diameter was divided by pulse pressure to derive the distensibility index. Traditionally, measures of arterial distensibility have been analyzed as change in diameter during the cardiac cycle, adjusted for lumen diameter and pulse pressure. The distensibility index is given by the equation:

$$(((d_2\text{-}d_1)/d_1)*100)/(\text{SBP-DBP})$$

in which d_1 is the minimum carotid diameter, d_2 is the maximum carotid diameter, SBP is systolic blood pressure, and DBP is diastolic blood pressure.

Reproducibility

Eleven control subjects attended on 2 separate occasions for ultrasound measurements and measurements of blood pressure so that reproducibility of percentage increase in carotid luminal diameter measurements and of the distensibility index could be assessed. The coefficient of variation for measurements of percentage increase in carotid luminal diameter was 24%, pulse pressure was 13%, and the distensibility index was 31%.

Statistics

To compare a continuous variable between the 2 groups, the unpaired *t* test or the Mann-Whitney test was used for variables with normal and skewed distributions, respectively. Continuous variables with normal distributions are expressed as mean with standard deviations; continuous variables with skewed distributions are expressed as median with interquartile ranges. To compare categorical variables between the 2 groups, Fisher's exact test was performed.

Linear regression was used to assess the relationship between continuous variables within a group. Backward stepwise multiple regression was used to assess the relationship between a continuous variable and more than 1 continuous or categorical variables within a group. Analysis of covariance was used to assess whether relationships between 2 continuous variables differed between type 1 diabetic and nondiabetic subjects (diabetic status was the covariate). For all regression analyses, residual analyses were performed to assess the validity of the model.

The Arcus Quickstat Biomedical package was used for the analyses (Longman Software Publishing, Cambridge, UK).

RESULTS

Forty-one type 1 diabetic subjects and 50 nondiabetic subjects were recruited. Table 1 shows the demographic and clinical characteristics of both groups. While both groups had similar sex distributions and body mass indices, the diabetic subjects were significantly older (39 [29 to 52] *v* 32 [28 to 36] years; $P = .015$). In the diabetic group, HbA_{1c} was 7.63% (1.17), and duration of diabetes was 12 (0 to 23) years. Four diabetic subjects (10%) were known to have coronary heart disease, 22 (54%) had retinopathy (at least background), and 14 (34%) had microalbuminuria. Fasting blood for glucose and HbA_{1c} were taken from all diabetic subjects, and fasting blood for glucose was taken from 25 nondiabetic subjects. Table 1 also shows the biochemical and ultrasonic characteristics of both groups.

Serum VEGF concentrations were significantly higher in the type 1 diabetic subjects compared with nondiabetic subjects (217 [135 to 336] *v* 137 [80 to 237] pg/mL; $P = .009$). SBP was significantly higher in the type 1 diabetic compared with non-

Table 1. Demographic, Clinical, Biochemical, and Ultrasonic Characteristics of Subjects

	Type 1 Diabetic Subjects	Nondiabetic Subjects	*P* Value
No.	41	50	
Sex: no. male (%)	26 (63)	27 (54)	.399
Body mass index (kg/m^2)	24.9 (3.0)	24.7 (3.8)	.827
Age (yr)	39 (29-52)	32 (28-36)	.015*
Duration of diabetes (yr)	12 (0-23)		
Retinopathy: no. (%)	22 (54)		
Microalbuminuria: no. (%)	14 (34)		
Coronary heart disease: no. (%)	4 (10)	0 (0)	.052
SBP (mm Hg)	130 (120-148)	121 (114-128)	.002*
DBP (mm Hg)	74 (9)	75 (8)	.584
Pulse pressure (mm Hg)	55 (45-71)	46 (41-51)	.0003*
HbA_{1c} (%)	7.63 (1.17)		
Fasting glucose (mmol/L)	10.7 (8.4-15.5)	4.8 (4.4-5.3)	<.0001*
VEGF (pg/mL)	217 (135-336)	137 (80-237)	.009*
Mean carotid diameter (mm)	5.29 (0.59)	5.11 (0.66)	.190
Mean increase in carotid luminal diameter (%)	12.9 (10.2-15.7)	13.0 (10.6-15.0)	.864
Distensibility index (%/mm Hg)	0.2439 (0.1024)	0.2855 (0.0792)	.031*

NOTE. Categorical variables (eg, sex) are expressed as number (and the percentage) in a category; continuous variables (eg, body mass index) are expressed as mean (and the standard deviation) if the variable has a normal distribution or as median (and the interquartile range) if the variable has a skewed distribution.

* $P < .05$.

diabetic subjects (130 [120 to 148] *v* 121 [114 to 128] mm Hg; $P = .002$).

The type 1 diabetic group had a significantly lower distensibility index (0.2439 [0.1024] *v* 0.2855 [0.0792]%/mm Hg; $P = .031$). Mean carotid diameter was similar in the 2 groups (5.29 [0.59] *v* 5.11 [0.66] mm; $P = .190$). The percentage increase in carotid luminal diameter during the cardiac cycle was not significantly different in the 2 groups (12.9% [10.2 to 15.7] *v* 13.0% [10.6 to 15.0]; $P = .270$), despite significantly greater pulse pressure in the type 1 diabetic group (55 [45 to 71] *v* 46 [41 to 51] mm Hg; $P = .0003$).

There was a significant negative correlation between serum VEGF concentrations and mean percentage increase in carotid luminal diameter during the cardiac cycle in the diabetic group ($r = -.36$, $P = .021$) and in the nondiabetic group ($r = -.28$, $P = .047$). Significant vertical separation of the regression lines accounted for the higher serum VEGF concentrations in the diabetic subjects (analysis of covariance, $P = .023$) (Fig 1). This negative correlation could be strengthened by relating mean percentage increase in carotid luminal diameter to pulse pressure to give the distensibility index. Therefore, serum VEGF concentrations correlated strongly and inversely with the distensibility index in the diabetic group ($r = -.49$, $P = .001$) (Fig 2A) and in the nondiabetic group ($r = -.29$, $P = .041$) (Fig 2B) and in both groups analyzed together ($r = -.42$, $P < .0001$). The slopes of the regression lines for the 2 groups were not significantly different, and there was no significant vertical separation of the regression lines.

In the type 1 diabetic subjects, serum VEGF concentrations correlated significantly with SBP ($r = .34$, $P = .028$) and mean arterial pressure (MAP) ($r = .34$, $P = .030$), but not with DBP ($r = .26$, $P = .10$) or pulse pressure ($r = .28$, $P = .08$). There were no correlations between serum VEGF concentrations and any measures of blood pressure in the nondiabetic group. If both groups were analyzed together, serum VEGF concentrations correlated significantly with SBP ($r = .28$, $P = .008$), MAP ($r = .24$, $P = .022$) and pulse pressure ($r = .23$, $P = .027$), but not with DBP ($r = .13$, $P = .216$).

SBP correlated with distensibility index in the diabetic group ($r = -.64$, $P < .0001$), in the nondiabetic group ($r = -.62$, $P < .0001$) and in both groups analyzed together ($r = -.62$, $P < .0001$).

Serum VEGF concentrations correlated significantly with age in the nondiabetic group ($r = .50$; $P = .0002$) and in both groups analyzed together ($r = .36$, $P = .0004$), but not in the type 1 diabetic group ($r = .21$, $P = .191$). Age correlated with distensibility index in the diabetic group ($r = -.66$, $P < .0001$),

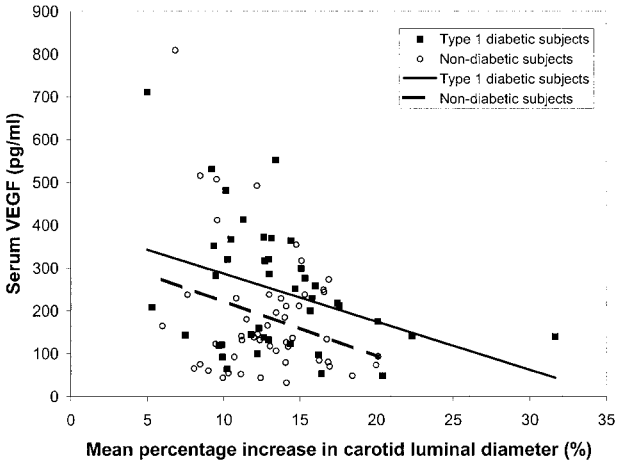

Fig 1. Serum VEGF concentration *v* percentage increase in carotid luminal diameter in type 1 diabetic subjects ($r = -.36$, $P = .021$) and nondiabetic subjects ($r = -.28$, $P = .047$). The vertical separation of the regression lines is significant (analysis of covariance, $P = .023$).

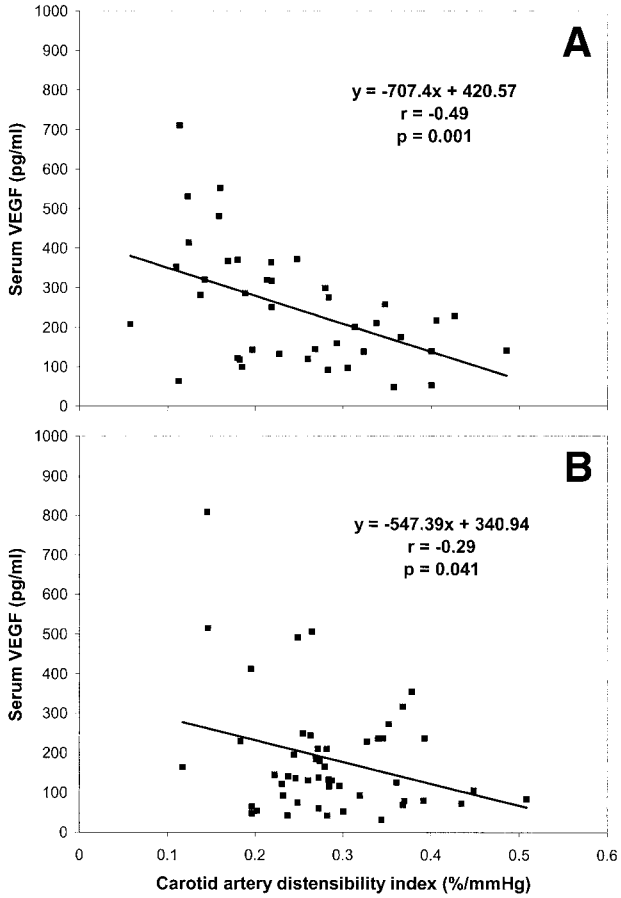

Fig 2. Serum VEGF concentration *v* carotid artery distensibility index in type 1 diabetic subjects (A) and in nondiabetic subjects (B). There was no significant difference in either the slopes or the vertical separation of the regression lines for the 2 groups (analysis of covariance).

in the nondiabetic group ($r = -.75$, $P < .0001$), and in both groups analyzed together ($r = -.46$, $P = .0008$).

Although serum VEGF concentrations were significantly higher in the type 1 diabetic subjects, there were no significant correlations between serum VEGF concentrations and HbA_{1c}, fasting glucose concentrations or duration of diabetes. As the type 1 diabetic subjects had higher SBPs, were older, and had lower carotid artery distensibility indices, a backward stepwise multiple regression model was constructed, considering both groups together, with serum VEGF concentration as the dependent variable, and diabetic status, age, SBP, and distensibility index as the predictors. Distensibility index was the only significant independent predictor of serum VEGF concentrations.

DISCUSSION

This study has demonstrated a strong inverse association between serum VEGF concentrations and carotid artery distensibility in both type 1 diabetic subjects and nondiabetic control subjects. Although the study also demonstrated associations between serum VEGF concentrations and diabetic status, blood pressure and age, these associations were lost when controlled for arterial distensibility.

Although easily measured, there have been remarkably few studies of the value of blood measurements of VEGF in diabetic subjects. One of the earliest studies was in a small group of children.[10] In this group, there was no difference in VEGF concentrations between diabetic and nondiabetic subjects. The value of the study was, however, limited by the small number of subjects involved. In a larger group of adults, a greater proportion of diabetic subjects than nondiabetic subjects were found to have detectable plasma VEGF concentrations, although this failed to achieve statistical significance (50% *v* 26%; $P = .084$).[11] In a more recent study, again in children, diabetic subjects had significantly higher serum VEGF concentrations than nondiabetic subjects.[12] In this study, there was an apparent relationship between the degree of glycemic control and serum VEGF concentrations. A subgroup of children followed prospectively for 2 years subsequently demonstrated a reduction in serum VEGF concentrations with improved glycemic control. There was no relationship between VEGF and blood pressure in the children. In an adult population, the situation is likely to be more complicated. In our previous study of a mixed population of diabetic adults, there was no difference between serum VEGF concentrations in diabetic and nondiabetic subjects.[6] Although we were able to find a positive association between HbA_{1c} and VEGF, when blood pressure was taken into account, the association was lost, so that measures of blood pressure were the major determinants of VEGF in this group.

In the present study, we have found a difference between serum VEGF concentrations in the type 1 diabetic subjects and the nondiabetic control subjects. The diabetic subjects had a high prevalence of microvascular complications, and this factor may have contributed to the higher VEGF concentrations in the diabetic group. As in our previous work, there was no relationship with glycemic control, but the relationship with blood pressure was again apparent in the diabetic group. The relationship between serum VEGF concentrations and blood pressure had formed the basis of the study. If there is indeed a relationship between serum VEGF and blood pressure, by what mechanism is it mediated? By examining ultrasonic characteristics of the study population, we hoped to gain some insight into the mechanisms.

The fact that reduced carotid artery distensibility is associated with higher serum VEGF concentrations suggests that mechanical stretch of vessel walls is not an important determinant of serum VEGF concentrations, because reduced vessel distensibility implies less mechanical stretch per heart beat. In vitro exposure of human vascular smooth muscle cells to cyclical mechanical stretch significantly increases VEGF messenger RNA and peptide production,[7] and myocardial stretch in an isolated perfused rat heart has been shown to increase VEGF expression.[8] These in vitro findings cannot explain our observations, so that in vivo, other factors may override the influence of vascular smooth muscle stretch. Therefore, although we had hypothesized that greater vascular stretch would be associated with higher serum VEGF concentrations, we, in fact, demonstrated the opposite.

Decreased arterial distensibility is associated with greater

vessel wall shear stress and greater flow velocity. This suggests that shear stress and flow velocity may be major determinants of serum VEGF concentrations. The source of the circulating VEGF is not clear, but potential sources are vessel wall constituents, such as endothelial or smoooth muscle cells[13] or cellular components of blood, such as leukocytes[14] or platelets.[15]

The current literature suggests that glycemic control may be an influence on circulating VEGF levels in diabetic subjects. We would suggest based on the present study that, at least in adult populations, vessel wall distensibility, and so shear stress and flow velocity, may be more important factors in both diabetic and nondiabetic populations and may underlie the association between blood pressure and serum VEGF concentrations that we have previously observed. The pathophysiologic relevance of such factors remains to be elucidated.

REFERENCES

1. Aiello LP, Avery RL, Arrigg PG, et al: Vascular endothelial growth factor in ocular fluid of patients with diabetic retinopathy and other retinal disorders. N Engl J Med 331:1480-1487, 1994

2. Tanaka Y, Katoh S, Hori S, et al: Vascular endothelial growth factor in diabetic retinopathy. Lancet 349:1520, 1997

3. Aiello LP, Pierce EA, Foley ED, et al: Suppression of retinal neovascularization in vivo by inhibition of vascular endothelial growth factor (VEGF) using soluble VEGF-receptor chimeric proteins. Proc Natl Acad Sci USA 92:10457-10461, 1995

4. Adamis AP, Shima DT, Tolentino MJ, et al: Inhibition of vascular endothelial growth factor prevents retinal ischaemia-associated iris neovascularization in a nonhuman primate. Arch Ophthalmol 114:66-71, 1996

5. Couffinhal T, Kearney M, Witzenbichler B, et al: Vascular endothelial growth factor/vascular permeability factor (VEGF/VPF) in normal and atherosclerotic human arteries. Am J Pathol 150:1673-1685, 1997

6. Sharp P, Al-Mrayat M, Valabhji J, et al: Serum levels of vascular endothelial growth factor in diabetic subjects: The relationship with blood pressure. Diabetologia 41:984-985, 1998

7. Kimber P, O'Callaghan CJ, Williams B: Chronic cyclical mechanical stretch increses production of vascular permeability factor mRNA and peptide in human vascular smooth muscle cells. J Hypertens 14:S66, 1996

8. Li J, Hampton T, Morgan JP, et al: Stretch-induced VEGF expression in the heart. J Clin Invest 100:18-24, 1997

9. Hoeks APG, Brands PJ, Smeets FAM, et al: Assessment of the distensibility of superficial arteries. Ultrasound Med Biol 16:121-128, 1990

10. Malamitsi-Puchner A, Sarandakou A, Tziotis J, et al: Serum levels of basic fibroblast growth factor and vascular endothelial growth factor in children and adolescents with type 1 diabetes mellitus. Pediatr Res 44:873-875, 1998

11. Wasada T, Kawahara R, Katsumori K, et al: Plasma concentration of immunoreactive vascular endothelial growth factor and its relation to smoking. Metabolism 47:27-30, 1998

12. Chiarelli F, Spagnoli A, Basciani F, et al: Vascular endothelial growth factor (VEGF) in children, adolescents and young adults with type 1 diabetes: Relation to glycaemic control and microvascular complications. Diabet Med 17:650-656, 2000

13. Williams B: Factors regulating the expression of vascular permeability/vascular endothelial growth factor by human vascular tissue. Diabetologia 40:S118-S120, 1997

14. Webb NJ, Myers CR, Watson CJ, et al: Activated human neutrophils express vascular endothelial growth factor (VEGF). Cytokine 10:254-257, 1998

15. Weltermann A, Wolzt M, Petersmann K, et al: Large amounts of vascular endothelial growth factor at the site of hemostatic plug formation in vivo. Arterioscler Thromb Vasc Biol 19:1757-1760, 1999