Table 1. HFE Cys282Tyr polymorphism in patients with Type II diabetes and control subjects

nucl. 845 (amino acid)	a/a (tyr/tyr)	a/g (tyr/cys)	g/g (cys/cys)	Σ
Type II	1 (0.5 %)	17 (8.5 %)	177 (91 %)	195[a]
control subjects	0	26 (14 %)	154 (86 %)	180

[a] p = NS

Table 2. HFE His63Asp polymorphism in patients with Type II diabetes and control subjects

nucl. 187 (amino acid)	c/c (his/his)	c/g (his/asp)	g/g (asp/asp)	Σ
Type II	147 (71 %)	55 (27 %)	4 (2 %)	206[a]
control subjects	121 (69 %)	49 (28 %)	5 (3 %)	175

[a] p = NS

merase chain reaction (PCR) were constructed according to the published DNA sequence [1] (GenBank U630319): 5'-GGAGTTCGAACCTAAAGACGT and 5'AGGGCTCC-CAGATCACAATG for the Cys282Tyr mutation; 5'-TCA-GAGCAGGACCTTGGTCTT and 5'-ACTCTGACT-CAGCTGCAGCCA for the His63Asp mutation. Amplified fragments were purified and subsequently digested with RsaI (Cys282Tyr) respectively with DpnII (His63Asp).

The distribution of *HFE* genotypes did not differ significantly between patients and control subjects: Cys282Tyr heterozygosity occurred in 17 patients (8.5 %) and in 26 control subjects (14 %, Table 1). The His63Asp mutation was found in 55 patients (27 %) and 49 control subjects (28 %) in its heterozygous form (Table 2). These figures are in accordance with those published previously except for a slightly higher frequency of Cys282Tyr heterozygotes in control subjects. Other studies have found heterozygotes in frequencies varying from 5 to 10 per cent in control subjects derived from European populations [8, 9].

Our screening led to the identification of one diabetic patient who presented with high iron/ferritin levels at diabetes manifestation. He is homozygous for the Cys282Tyr mutation and liver biopsy confirmed the diagnosis of genetic haemochromatosis.

In conclusion, the *HFE* mutations Cys282Tyr and His63Asp do occur in similar frequencies as heterozygotes in patients with Type 2 diabetes mellitus and in control subjects. Heterozygosity for hereditary haemochromatosis mutations does not represent a genetic risk marker for Type 2 diabetes mellitus in our study group. However, when suspecting iron overload as a cause of secondary diabetes, the *HFE* mutation analysis can confirm the diagnosis of genetic haemochromatosis.

Yours sincerely,
J. Braun, H. Donner, K. Plock, H. Rau, K. H. Usadel, K. Badenhoop

References

1. Feder JN, Gnirke A, Thomas W et al (1996) A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. Nature Genetics 13: 399–408
2. Dinneen SF, Silverberg JD, Batts KP et al (1994) Liver iron stores in patients with non-insulin dependent diabetes mellitus. Mayo Clin Proc 69: 13–15
3. Edwards CQ, Griffen LM, Goldgar D et al (1988) Prevalence of haemochromatosis among 11 065 presumably healthy blood donors. N Engl J Med 318: 1355–1362
4. Dadone MM, Kushner JP, Edwards CQ, Bishop DT, Skolnick MH (1982) Hereditary haemochromatosis: analysis of laboratory expression of the disease by genotype in 18 pedigrees. Am J Clin Pathol 78: 196–207
5. Risch L (1997) Haemochromatosis, HFE and genetic complexity. Nature Genetics 17: 375–376
6. Cutler P (1989) Deferoxamine therapy in high-ferritin diabetes. Diabetes 38: 1207–1210
7. Nelson RL, Davis FG, Persky V, Becker (1995) Risk of neoplastic and other diseases among people with heterozygosity for hereditary haemochromatosis. Cancer 76: 875–879
8. Roberts AG, Whatley SD, Morgan RR, Worwood M, Elder GH (1997) Increased frequency of the haemochromatosis Cys282Tyr mutation in sporadic porphyria cutanea tarda. Lancet 349: 321–323
9. Gottschalk R, Seidl C, Löffler T et al (1998) HFE codon 63/282 dimorphism in German patients with genetic haemochromatosis. Tissue Antigens 51: 270–275

Serum levels of vascular endothelial growth factor in diabetic subjects: the relationship with blood pressure

Dear Sir,
Vascular endothelial growth factor is a cytokine whose main actions are to increase vascular permeability, and to induce angiogenesis [1]. Since these functions have parallels with the major manifestations of endothelial dysfunction in diabetes, this growth factor has provoked interest, particularly in respect of diabetic microvascular disease [2–4].

Corresponding author: Dr. P. S. Sharp, Level 9, Northwick Park and St Mark's NHS Trust, Watford Road, Harrow, Middlesex, HA1 3UJ, UK

It is now possible to measure vascular endothelial growth factor in blood. We therefore embarked upon pilot work to assess the value of such measurements in respect of clinical markers in diabetic subjects using a commercially available kit (R&D Systems Ltd. Abingdon UK). This is directed against the 165 amino acid isoform of vascular endothelial growth factor which is the most abundant circulating isoform in humans. During this work, we discovered an intriguing association with arterial blood pressure which we report here.

Sixty three diabetic subjects were recruited to the study [39 male, 24 female, 33 on insulin, age 51.3 ± 13.2 years (standard deviation), 32 with no retinopathy]. Subjects were selected with a minimum duration of diabetes of 5 years, to allow for the development of diabetic retinopathy. Those with a raised creatinine or any other significant medical condition were excluded. Non-diabetic subjects were recruited from amongst the staff at the Hospital (26 male, 22 female, age 38.8 ± 13.9 years). Serum vascular endothelial growth factor was

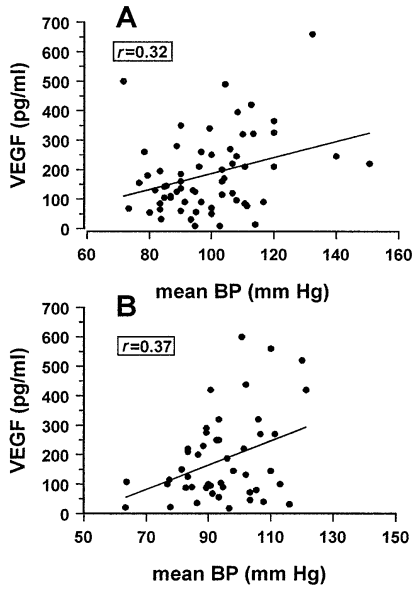

Fig. 1 A, B. Scatter plots and linear regression lines for serum VEGF against mean blood pressure for (**A**) diabetic and (**B**) non-diabetic subjects

184.9 ± 131.3 pg/ml in diabetic subjects compared with 185.0 ± 146.0 pg/ml in non-diabetic subjects. Amongst the diabetic subjects, there was no difference in serum vascular endothelial growth factor by treatment modality or sex, and no significant correlation with age, duration of diabetes, body mass index, creatinine, glycated haemoglobin or logged values for urinary albumin/creatinine ratio. Serum vascular endothelial growth factor levels were 170.7 ± 126.3 pg/ml in patients with no retinopathy, 150.5 ± 129.1 pg/ml in those with microaneurysms only and 188.2 ± 84.8 pg/ml in those with more advanced retinopathy (not significant on analysis of variance ($F = 0.4$, $p = 0.67$)).

The striking finding was the correlation between vascular endothelial growth factor and all measures of blood pressure; diastolic blood pressure ($r = 0.274$, $p < 0.05$), systolic blood pressure ($r = 0.313$, $p < 0.02$) and mean blood pressure ($r = 0.32$, $p < 0.02$). There was no correlation with pulse pressure. Amongst the non-diabetic subjects, very similar relationships were seen, although correlations were somewhat stronger due to fewer outlying values (serum vascular endothelial growth factor vs diastolic blood pressure, $r = 0.32$ [$p < 0.05$], systolic blood pressure, $r = 0.34$ [$p < 0.02$], mean blood pressure, $r = 0.37$ [$p < 0.01$]).

Regression of serum vascular endothelial growth factor on mean blood pressure in the diabetic subjects produced the equation of sVEGF = −84 + 2.7 mean blood pressure, $r^2 = 10.3\,\%$, $F = 6.7$, $p = 0.011$.

No relationship between serum vascular endothelial growth factor and arterial blood pressure has been shown previously. Such a relationship should not, however, be totally surprising. Vascular endothelial growth factor is expressed in a wide variety of cell lines, including vascular endothelium and smooth muscle cells [1]. A variety of stimuli have been shown to upregulate expression in vascular tissues, most notably hypoxia and high glucose concentrations [3]. In addition, chronic cyclical mechanical strain has been shown to increase mRNA production and peptide production in vascular smooth muscle cells [5]. Other studies have demonstrated that in the rat heart, dilatation of the ventricle increases vascular endothelial growth factor expression in the myocardium [6, 7]. It is thus possible that the increased mechanical strain associated with hypertension upregulates vascular endothelial growth factor in the vascular wall, the secreted isoforms of which are measurable in serum. The fact that there was no correlation with HbA_1, and that relationships with blood pressure seem to apply equally in diabetic and non-diabetic subjects suggests that this is not a diabetes related phenomenon, the cause lying purely with the dynamics of increasing blood pressure. Furthermore, the fact that the relationship is stronger with measures reflecting the pressure within the vasculature, but not with pulse pressure, might support the hypothesis proposed by others [5–7] that it is vascular strain and not mechanical stress that is the responsible factor.

We conclude that blood measurements of vascular endothelial growth factor merit further study, particularly in relation to blood pressure. That there was no relationship between blood levels of vascular endothelial growth factor and clinical evaluations of diabetic retinopathy may reflect the known lack of correlation between levels in eye and serum [8]. Additionally, the standard deviations of serum vascular endothelial growth factor levels demonstrated in the present study indicate that a considerably larger sample size would be necessary to make any definitive statement.

Yours sincerely,
P. S. Sharp, M. Al-Mrayat, J. Valabhji, T. M. Kearney, D. Wright

Acknowledgement. This work was supported by a grant from Eli Lilly Co. Ltd.

References

1. Dvorak HF, Brown LF, Detmar M, Dvorak AM (1995) Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis. Am J Pathol 146: 1029–1039
2. Aiello LP, Avery RL, Arrigg PG et al. (1994) Vascular endothelial growth factor in ocular fluid of patients with diabetic retinopathy and other retinal disorders. N Engl J Med 331: 1480–1487
3. Williams B (1997) Factors regulating the expression of vascular permeability/vascular endothelial growth factor by human vascular tissues. Diabetologia 40 [Suppl 2]: S118–S120
4. Williams B (1996) Vascular permeability/vascular endothelial growth factors: a potential role in the pathogenesis and treatment of vascular diseases. Vascular medicine 1: 251–258
5. Kimber P, O'Callaghan CJ, Williams B (1996) Chronic cyclical stretch increases production of vascular permeability factor mRNA and peptide in human vascular smooth muscle cells. J Hypertension 14: S206
6. Li J, Brown LF, Hibberd MG, Grossman JD, Morgan JP, Simons M (1996) VEGF flk-1, flt-1 expression in a rat myocardial infarction model of angiogenesis. Am J Physiol 270: H1803–H1811
7. Li J, Hampton T, Morgan JP, Simons M (1997) Stretch-induced VEGF expression in the heart. J Clin Invest 100: 18–24
8. Burgos R, Simo R, Audi L et al. (1997) Vitreous levels of vascular endothelial growth factor are not influenced by its serum concentrations in diabetic retinopathy. Diabetologia 40: 1107–1109