Vascular endothelial growth factor insertion/deletion gene polymorphism in West Indian patients of type 2 diabetes and diabetic nephropathy

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Diabetic nephropathy (DN) is a major complication of diabetes mellitus (DM) and an important cause of end-stage renal failure requiring dialysis or renal transplantation. The risk of nephropathy is strongly determined by genetic factors and there is greater possibility that patients with either type 1 or type 2 diabetes will ultimately develop DN1. Evidence of an important genetic component of DN has stimulated extensive efforts to decipher the genetic architecture of disease in multiple populations. DN susceptibility loci can be identified in a variety of ways. Three broad analytic approaches have typically been applied: candidate gene studies, linkage analysis and genome-wide association studies, each identifying different putative susceptibility loci2. The candidate gene approach involves assessment of genetic variation, typically single nucleotide polymorphisms (SNPs) in one or more genes with plausible physiological roles in DN3.

Vascular endothelial growth factor (VEGF) is a potent multi-functional cytokine which plays a key role in the pathogenesis of diabetic microvascular complications4,5. Endothelial dysfunction and increased blood vessel permeability have been observed in both diabetic retinopathy and DN6. The VEGF is a highly conserved homodimeric glycoprotein which promotes angiogenesis and is a potent mediator of microvascular permeability7. The genetic variations in the VEGF gene influence levels of VEGF protein expression. There are several polymorphisms in the VEGF gene and many polymorphisms are associated with the protein production. Among these, four VEGF SNPs, namely +936C/T in the 39-untranslated region, –634G/C in the 59-untranslated region and –2578C/A and –1154G/A in the promoter region have been reported to modulate VEGF expression8-11.

Although it is reported that there is lack of association of the VEGF gene polymorphisms with diabetic retinopathy in South Indian population12, but no study has been done so far on role of the VEGF gene polymorphism in DN in Indian population. In this study, we have aimed to determine the possible I/D polymorphism in the gene encoding the VEGF in diabetic patients and patients having DN in West Indian population.

Materials and Methods

Participants
A total of 456 patients with type 2 diabetes, nephropathy with diabetes, nephropathy without...
diabetes and healthy control were considered for the study. Patients with concurrent acute illnesses including infectious diseases within the past 1 week, malignancy and active immunological diseases, medical history of clinical cardiovascular disease, confounding factors for proteinuria, such as severe uncontrolled hyper tension (> 160/100 mm Hg) or renal insufficiency (serum creatinine > 1.5 mg/dL) and smoking history were excluded from the study. Informed consent was obtained from participants after a clear explanation of potential risk of the study.

This study was comprised in four groups: i) healthy control (n = 143), ii) type 2 diabetes (n = 103), iii) diabetic nephropathy (n = 102) and iv) non-diabetic nephropathy (n = 108). Diagnosis of DM was based on the recommendation of American Diabetes Association. Duration of diabetes was considered as the time from which the patient was diagnosed with diabetes. Nephropathy was diagnosed on the basis of persistent microalbuminuria (Urinary albumin excretion rate (UAER) 20–200 mg/24 h) or proteinuria (UAER > 200 mg/24 h) in diabetic and non-diabetic participants by the consulting physician. The detailed medical and clinical demography, including height, weight, duration of diabetes, age and medication were obtained. After explaining the purpose of this study, signed informed consent forms were obtained from the participants.

Blood samples were collected in EDTA coated Vacutainer™ tubes and plasma was separated from blood cells by centrifuging at 3,000 rpm for 10 min. The serum creatinine and glucose concentration was determined using commercially available enzymatic kits using the modified Jaffe's and GOD/POD method respectively. Glycosylated hemoglobin was determined by nyco card reader.

**Determination of VEGF genotypes**

DNA was isolated from collected blood sample by Nonidet method13. The I/D polymorphism was analysed using the following primers: forward 5'-GCTGAGAGTGGGGCTGACTAGGTA-3' and reverse 5'-GTTTCTGACCTGGCTATTTCCAGG-3'. Genomic DNA was amplified by PCR in a final volume of 30 µl using the following conditions: denaturation at 95°C for 6 min, followed by 35 cycles at 94°C for 1 min, 57°C for 1.5 min and 72°C for 2 min and a final extension at 72°C for 10 min. Reaction products were analysed by agarose gel electrophoresis. VEGF I/D polymorphism was shown by the presence of different bands: 211bp (D allele) and 229 bp (I allele) (Fig. 1).

![Fig. 1—PCR amplicon of VEGF gene (I/D) polymorphism (M, 100 bp marker; II, 229 bp; ID, 229 and 211 bp; and DD, 211 bp; lanes 1,3,4,5,6,7,8,9,11, ID genotype; lane 12, DD genotype; and lane 2, 10, II genotype)](image)

**Statistical analysis**

Statistical analyses were performed using SPSS 15.0 for Windows. Data are presented as mean ± standard deviation (SD). Genotype distribution, allele frequencies and allele positivity were assessed by χ² test of independence with 3 × 2 and 2 × 2 contingency tables and Z-statistics. Fisher’s exact test was used to analyze allele frequencies and allele positivity in the various patient studied groups. ANOVA test was used to compare different means. Values of P< 0.05 were considered statistically significant. Odds ratios (OR) with 95% confidence intervals (CI) were estimated for the effects of high risk alleles.

**Results**

The clinical characteristics and demographic details of control, T2DM, DN and NDN participants are summarized in Table 1. The mean age of the control participants (47 ± 11.2 yrs) was non-significantly lower than the T2DM (55 ± 7.3 yrs) or DN (59 ± 12.5 yrs) or NDN (58 ± 12.3 yrs). There was no significant difference observed in body mass index (BMI), systolic and diastolic blood pressure and hemoglobin levels in all the three studied groups (T2DM, DN and NDN) compared to control participants and also between three different studied groups (T2DM, DN and NDN). However, glycosylated haemoglobin (HbA1c) in type 2 diabetic patients without any complications and in patients with DN were significantly (p < 0.05) higher than control healthy participants. Serum creatinine level was significantly higher among patients with NDN and DN, compared to T2DM and control participants.

The genotypic and allelic frequencies of VEGF (I/D) gene in T2DM, DN, NDN patients and control participants are shown in Table 2. There was significant increase in ID genotype in all groups, compared to II and DD genotypes. No significant
association of I or D allele was observed in NDN, compared to control (p = 0.24) in case of DM (p = 0.78) and DN (p = 0.52). Also, there was no significant association of II genotype in NDN, compared to DM (p = 0.82).

The odd ratio (OR) for D allele vs I allele between DM with DN was 0.547 (95% CI 0.54–1.23) with p = 0.82 and DM with NDN was 0.922 (95% CI 0.694–1.54) with p = 0.92. The odd ratio for D allele vs I allele between control with DM, DN and NDN was 1.06 (95% CI 0.73–1.55) with p = 0.78; 0.87 (95% CI 0.60–1.28) with p = 0.52; 1.10 (95% CI 0.76–1.60) with p = 0.58, respectively (Table 3).

**Discussion**

We studied various anthropogenic parameters and found that creatinine level was significantly higher in DM and NDN groups, compared to control group. The relationship between HbA1c, blood glucose concentrations and late complications has been reported earlier. We found that HbA1c level was significantly higher in DM and DN groups, which was due to uncontrolled hyperglycemic condition. In present study, there was no significant difference observed in BMI and blood pressure between control and patients’ group.

Polymorphism in VEGF has been reported to have an effect on the regulation of gene expression that results in altered levels of VEGF and may, therefore, contribute to the pathogenesis of many diseases. Haplotype -460C/+405G has a higher promoter activity than haplotype -460T/+405C. For 936C>T polymorphism, 936T allele is also reported to be associated with lower VEGF plasma levels. However, in a Japanese study, no association has been observed.
found between 936 T polymorphism and VEGF serum levels. The I/D polymorphism in the promoter region of the VEGF gene is suggested to be associated with retinopathy, but not nephropathy in DM patients in population of Poland in DD genotype. In Chinese population, significant increase in DD genotypes in patients with DN is reported, compared with those without complications (40.2% vs. 22.7%, respectively). We investigated the possible association I/D polymorphisms in VEGF gene with DN in patients with and without DM in Indian population. Our results showed that the frequency of ID genotype was higher, compared to II and DD genotypes in all the groups, which might reflect some cell-specific effects of VEGF polymorphism. These results differed from those obtained in another study, wherein an association is found between the DD genotype of the VEGF gene I/D polymorphism and an increase in the risk of diabetic retinopathy. The precise role of VEGF in DN is still uncertain. It is not known whether expression of VEGF is, at least in part, the cause of pathological changes in nephropathy or rather represents a reparative response as a consequence of pre-existing tissue and functional alterations.

In conclusion, our study indicated that I/D polymorphism in the promoter region of the VEGF gene was not significantly associated with DN in Indian type 2 diabetic patients and increased creatinine level was found to be an independent risk factor in development of DN. However, further investigations with increased sample size are necessary to confirm the significant effect of the D allele of the VEGF gene on the development of DN.

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