Association between Vascular Endothelial Growth Factor (VEGF) +405 C/G Polymorphism and Hypertension in North West of Iran

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ABSTRACT: Blood pressure is one of the main factors in global mortality. People with systolic blood pressure above 140 mmHg and diastolic blood pressure above 90 mmHg are known as patients with hypertension. Complications of the hypertension are numerous including visual impairment as important complication and kidney, brain, and heart damages. If hypertension is not treated, is often fatal. Genetic plays a pivotal role in blood pressure regulation. Whereas hypertension is multifactorial and familial condition. However, little is known regarding the contribution of polymorphisms in VEGF gene to essential hypertension (EH). We aimed to investigate the association between +405 VEGF C/G single nucleotide polymorphism (SNP) and occurrence of EH in a population of 100 subjects including 50 normal and 50 patients with Eh. In this study, we evaluated triglyceride, blood sugar, cholesterol and body mass index of patients. Genotyping of +405 VEGF C/G (rs2010963) SNP was detected using polymerase chain reaction—restriction fragment length polymorphism (RFLP). Genotype frequencies of GG, GC and CC were respectively 44%, 44% and 12% in hypertensive patients and 16%, 34% and 50% in the control group. Allele frequency of G and C were respectively, 66% and 34% of hypertensive patients and 33% and 67% in the control group. In this study, was found significant difference between the frequencies and this result was that the +405 VEGF C/G polymorphism is associated with blood pressure.

Keywords: polymorphism, VEGF gene, hypertension, single nucleotide polymorphism

INTRODUCTION

Essential hypertension (EH known as primary or idiopathic hypertension) comprises 95% of all cases of hypertension, and by itself, accounts for 7.1 million deaths from ischemic heart disease and stroke annually [2, 3]. Because of the high prevalence of hypertension among individuals and significant impact of genetic on this disease, numerous studies and researches on the relationship of genes with this disease have been made. There is some evidence to suggest that genetic factors contribute to blood pressure and hypertension.

Firstly, the normal distribution of blood pressure in the population indicates impact of environmental and genetic factors. Secondly, rare monogenic forms of hypertension associated with major defects in renal salt handling prove that gene mutations may contribute to the common essential hypertension [Hastie et al., 2010]. Both the Human Genome Project (HGP) and recent advances in biotechnology have made it possible for scientists to reveal the genes affiliated with susceptibility to EH and several studies using genome-wide mapping have disclosed many chromosomal regions propounded to be linked to EH [3, 5–7]. Two of these regions are chromosome 6q2 and 6p and a gene worth pondering is Vascular Endothelial Growth Factor A (VEGF-A) that locates at 6p21.3 [8]. This gene is a member of the PDGF/VEGF growth factor family and encodes a protein that is often found as a disulfide linked homodimer. This protein is a glycosylated mitogen that specifically acts on endothelial cells and has various effects, including mediating increased vascular permeability, inducing angiogenesis, vasculogenesis and endothelial cell growth, promoting cell migration, and inhibiting apoptosis [Claudio J. Conti, 2002]. Seven members of the VEGF family have been identified: VEGF-A, -B, -C, -D, -E and Placental Growth Factors (PIGF) 1 and 2. VEGF mediates its effects through VEGF receptors (VEGFR-1, VEGFR-2 and VEGFR-3) [Emily S. Robinson et al., 2011]. This gene has a 14-kbp coding region which consists of 8 exons and 7 introns [9]. It can be accompanied by polymorphisms, especially in the promoter region [10]. Evidential data collected in different studies has shown a correlation among specific VEGF-A SNPs with diseases and complications like coronary atherosclerosis, diabetic retinopathy and pre-eclampsia [9, 11–13]. Interestingly, Abnormal regulation...
of VEGF-A due to polymorphism at position -116 might represent a genetic factor for increased VEGF-A production and microvascular complications in EH [10]. In another study, it was shown that the VEGF +405 C/G polymorphism associates with EH patients among patients with diabetes, with the G allele being represented more frequently by EH subjects (Hamedian et al., 2011). This investigation was conducted because of the limited study on the association between the VEGF +405 C/G (rs2010963) polymorphism status and presence of high blood pressure also helped to open new areas to treat high blood pressure such as genet and pharmacogenomic.

MATERIAL AND METHODS

Subjects

50 hypertensive patients (25 males and 25 females) and 50 healthy controls (25 males and 25 females) were studied with the relevant professionals. However, patients with secondary hypertension, type 2 diabetes, liver disorders, kidney and thyroid were excluded from this study. Prior entering the study, informed consent was obtained from each subject. Blood pressure was measured by sphygmomanometer. BMI (body mass index assessment of obesity), cholesterol, fasting plasma glucose (FPG) and triglycerides were measured according to standardized methods.

+405 C/G polymorphism genotyping

Genomic DNA for analysis was isolated from peripheral blood leukocytes using standard techniques [16]. Polymerase chain reaction—restriction fragment length polymorphism (PCR—RFLP) was employed to determine the +405 VEGF C/G (rs2010963) polymorphism status with technical specifications described by Awata et al. [11]. Primers to detect the +405 VEGF C/G variant were Forward: 5’-TTGCTTGCCATTCCCCCTTGA-3’, Reverse: 5’-CGAAGCGAGAACAGCCAGAA-3’. PCR was performed in a volume of 10 µL including 100 ng DNA, 25 µL Taq PCR master mix RED, 2.5 µL primers, and 12.5 µL deionized water. Solutions were held in 95°C (5 min) for denaturation, followed by 35 cycles of 94°C (for denaturation; 1 min), 58°C (for annealing; 1 min), and 72°C (for extension; 1 min). An additional final extension of 72°C (5 min) was also performed. Final PCR product was digested using BsmFI restriction enzyme (biolabs). Electrophoresis was performed on agarose gel stained with 1% ethidium bromide. The C allele remained uncut, while the G allele was cut into two 195 and 274 bp fragments.

Statistical analyses

Statistical analyses were done using SPSS version 17.0 for windows. Categorical variables are expressed as proportions (%), and data were expressed as mean±standard deviation. Clinical characteristics of patients and controls were compared by testing T-TEST. The Chi-square test was used to compare the frequency of genotypes and alleles between hypertensive patients and controls. Logistic regression to investigate the risk factor of hypertension including age, body mass index (BMI), Triglycerides (TG), cholesterol (CHO), fasting plasma glucose (FPG) and +405 VEGF G/C polymorphism, was used. Genotypes were then placed in univariate and multivariate logistic regression models to calculate odds ratios (OR) with 95% confidence intervals (95% CI) for each genotype in predicting presence or absence of EH. A p-value less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

The clinical characteristics of hypertensive patients and healthy controls are shown in Table 1. Systolic and diastolic blood pressure, BMI, Triglycerides and cholesterol between the two groups of patients and healthy blood pressure showed significant differences. Despite the age, blood sugar with p-value more of 0.05 were not considered statistically significant. The DNA fragment of the VEGF gene was 469 bp after PCR amplification. If the PCR product include C polymorphism are not cut and segment remains with 469 bp length, but if include G polymorphism, yielded two fragments of 274 and 195 bp after digestion with BSMFI. Homozygous CC, without a restriction site only had a fragment of 469 bp. Homozygotic variants (GG) containing a restriction site in each DNA chain yielded two fragments of 274 and 195 bp after digestion with HapII. Heterozygote (GC) containing restriction sites in one of the DNA chains yielded 3 fragments of 469, 274 and 195 bp.
Genotype frequencies of GG, GC, and CC, respectively, 44%, 44% and 12% in hypertensive patients and 16%, 34% and 50% in the control group. Allele frequency of G and C, respectively, 66% and 34% of hypertensive patients and 33% and 67% in the control group (Table 2). Because the genotype and allele value less than 0.05, then the genotype and allele frequency significantly different in the two groups of patients and healthy. Logistic regression analysis showed that, in the additive model, +405 G/C VEGF associated with hypertension (OR: 0.298, CI: 0.164-0.541, p value: 0.000). The findings of logistic regression analysis indicated that age, BMI, TG, CHO and FPG were risk factors of hypertension (Table 3).

Clinical characteristics of population were studied and it was shown that cholesterol, triglycerides and BMI was significantly different between hypertensive and healthy individuals and people with high blood pressure have higher cholesterol, triglycerides and BMI. Logistic regression analysis revealed that age (p value: 0.000 OR: 1.022 CI: 1.012-1.033), cholesterol (p value: 0.000 OR: 1.005 CI: 1.004-1.005), fasting plasma glucose (p value: 0.003 OR: 1.005 CI: 1.002-1.009), triglycerides (p value: 0.000 OR: 1.019 CI: 1.018-1.020) and BMI (p value: 0.000 OR: 1.098 CI: 1.097-1.106) as a risk factor in the increased risk of high blood pressure in this study are considered. Each of these features increase the likely to develop hypertension increases.

The results created by our study pointed out a significant association between +405 C/G (rs2010963) VEGF gene SNP polymorphism and the presence of EH in patients. This SNP had a significant correlation with EH where G allele, and GG genotype compared CC genotype obtained higher probabilities to be carried by patients who had EH, suggesting that the G allele may increase the vulnerability of patients to EH. On the other hand, the +405 CC genotype was more likely to present itself in subjects that were not labeled hypertensive, establishing the hypothesis that the C allele could actually serve as a protective genetic factor against EH. These findings were in accordance with additive (G allele) and dominant (G and C allele dominant) inheritance models.

Since the discovery of VEGF (previously known as Vascular Permeability Factor; VPF), numerous studies have been conducted to clarify its physiological roles and highlight the possible pathophysiological consequences of the substance’s physiology [17–21]. Recently, a specific haplotype of the VEGF gene that was ‘C: 2578, G: 1154 and G: 634’, has been speculated to galvanize a specific ethnicity of white pregnant women against preeclampsia [13]. Various reports have linked +405 C/G SNP to specific pathologic conditions including lupus nephritis (GG genotype), collateral formation in coronary artery disease (CC genotype for better collateral formation), and heart failure (CC genotype for worse prognosis) [22–24]. A recent research demonstrated that the GG genotype of VEGF+405 C/G polymorphism is an independent predictor of albuminuria [25].
This research showed that the VEGF +405 C/G polymorphism associates with EH patients among patients with diabetes, with the G allele being represented more frequently by EH subjects. But we did not confirm the effects of the +VEGF polymorphism on the risk of hypertension and additional studies should be performed to clarify the contribution of variations of this gene to the pathogenesis of hypertension in a variety of ethnic groups. Hopefully, this scientific endeavor will disclose other scientists a different perspective of VEGF biology and EH and help open novel realms regarding therapeutic aspects of EH, such as gene therapy and pharmacogenomics.

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REFERENCES


