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Research paper

Association of a Vascular Endothelial Growth Factor genetic variant with Serum VEGF level in subjects with Metabolic Syndrome☆

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ABSTRACT

Background: The metabolic syndrome (MetS) is a clustering of metabolic disorders that is associated with an increased risk of developing cardiovascular-disease, diabetes, and related diseases. Against this background, Vascular Endothelial Growth Factor (VEGF) plays an essential role in angiogenesis, vascular permeability, and hematopoiesis and its increased level is reported to be associated with increasing the risk of developing cardiovascular-disease, stroke and diabetes. Therefore the aim of present study was to explore the association of serum VEGF level and its associated genetic-polymorphism, rs10738760 (A>G) at 9p24.2, in 850 subjects with/without MetS.

Methods: MetS was defined according to the International-Diabetes-Federation criteria. Genotyping was carried out using Polymerase chain reaction-amplification refractory mutation system. Anthropometric/biochemical parameters, including FBG, Triglyceride, HDL, TC, etc., were determined followed by univariate and multivariate analyses.

Results: MetS patients had significantly higher levels of BMI, waist-circumference, cholesterol, triglyceride, Hs-CRP and SBP/DBP, while the HDL-C levels was lower in patients group, compared to control group ($P < 0.05$). Moreover, our analysis showed that MetS patients with GA or AA genotypes had a significantly ($P = 0.03$) higher serum level of VEGF.

Conclusions: we demonstrate an association between a VEGF genetic variant with MetS, suggesting its role as a risk stratification factor for MetS.

Abbreviations: metabolic syndrome, (Mets); Vascular Endothelial Growth Factor, (VEGF); International Diabetes Federation, (IDF); chronic kidney diseases, (CKD); WC, waist circumference; TC, total Cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; FBG, fasting blood glucose; HC, hip circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure.

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1. Introduction

Metabolic syndrome (MetS) is defined as a clustering of metabolic disorders that include hypertension, dyslipidemia, central adiposity, and glucose abnormalities. It is associated with impaired angiogenesis, endothelial dysfunction, and a proinflammatory and prothrombotic state in the vasculature. These risk factors lead to an increased risk for insulin resistance, diabetes melitus, and cardiovascular diseases (CVD) (Ford et al., 2010; Zimmet et al., 1999; Grundy et al., 2006; Isomaa et al., 2001). The excessive prevalence of the MetS is reported to involve with the increasing prevalence of obesity and diabetes (Grundy et al., 2006; Isomaa et al., 2001). Several factors are associated with the development of MetS, environmental factors (e.g., lifestyle, gender, ethnicity,) and genetic factors (e.g., genetic polymorphisms in VEGF gene) (Mirhafez et al., 2015a).

Vascular endothelial growth factor (VEGF) is a multifunctional cytokine that plays a key role in many physiological (angiogenesis, growth and organ repair) and pathological (vascular disease) processes (Ferrara et al., 2003; Debette et al., 2011). Recently, a genome-wide association study identified two of the main single nucleotide polymorphisms implicated in VEGF gene (SNPs; rs6921438 and rs10738760) and explaining nearly half of the variance in serum VEGF levels (Debette et al., 2011). Rs10738760 is located on chromosome 9p24.2, between *VLDLR* and *KCNV2* genes that encode lipoprotein receptor and potassium voltage-gated channel subfamily V, member 2, respectively. A few studies have assessed the relationship between circulating VEGF levels and genetic polymorphisms (Jialal et al., 2010; Lieb et al., 2009a; Kraja et al., 2011a; Kristiansson et al., 2012a; Zabaneh and Balding, 2010a). In particular Debette et al., showed the important value of rs10738760 as well as its association with VEGF level (Debette et al., 2011). Therefore, the aim of current study was to investigate the associations of this genetic polymorphism with MetS for the first time in an Iranian population with and without metabolic syndrome.

2. Material and method

2.1. Phenotypic definition of MetS

MetS was defined according to the International Diabetes Federation (IDF) criteria: central obesity (defined as waist circumference of ≥94 cm for male or ≥80 cm for female) plus any two of the following four factors: elevated TG: ≥150 mg/dl (1·7 mmol/l); decreased HDL-cholesterol: <40 mg/dl (1·03 mmol/l) in males ,<50 mg/dl (1·29 mmol/l) in females; elevated systolic blood pressure (SBP) ≥130 or diastolic blood pressure (DBP) ≥85 mm Hg; elevated fasting blood glucose ≥100 mg/dl (5·6 mmol/l) (Zomorrodian et al., 2015).

2.2. Study participants

Eight hundred and fifty subjects were recruited from Mashhad University of Medical Science (MUMS). Individuals with known acute or chronic diseases such as stroke, myocardial infarctions, diabetes mellitus or cancer were excluded. Informed consent was obtained from all participants using protocols approved by the Ethics Committee of the Mashhad University of Medical Sciences (Zomorrodian et al., 2015).

2.3. Anthropometric and biochemical measurements

Anthropometric parameters (e.g., height, body weight, waist and hip circumference) were measured as described previously (Mirhafez et al., 2015b). BMI was calculated as body weight (kg) divided by squared height in meters (m^2), and BMI of 20–24·9, 25–29·9 and ≥30 kg/m^2 were considered as normal, over-weight or obese, respectively (Emamian et al., 2015). SBP and DBP (SBP or DBP) were measured. Total cholesterol, HDL, LDL and TG, CRP and fasting blood glucose (FBG) concentrations were assayed as described previously (Oladi et al., 2015; Mirhafez et al., 2015c).

2.4. DNA isolation and genotyping

Genomic DNAs from the Peripheral blood were extracted using Parstous Blood DNA Extraction Kit and QIAamp® DNA Mini-Kit (Qiagen, San Diego,CA) according to the manufacturer's protocol at Mashhad University of Medical Science and VU University Medical Center Amsterdam, respectively (Avan et al., 2013). Genotyping of VEGF gene SNP rs10738760 was performed using Polymerase chain reaction-amplification refractory mutation system (ARMS-PCR), as describe recently (Mirhafez et al., 2015b). The sequences of primers were: Wild type forward primer: 5-3 GATGGAAGGAAGTTGGGTG, Mutant forward primer: 5-3 GATGGAAGGAAGTTGGGTA, reverse primer: 5-3 ACTGTGTGC CTGTCCTTTAT. Hardy-Weinberg equilibrium was tested. The reaction was performed in 20 μl total volume, using 2 μl buffer, 1.6 μl dNTPs, 2 μl MgCl2, 10 pmol for each forward and reverse primers, 0.2 μl Taq Polymerase, and 10–20 ng/ul DNA. PCR system Veriti 96 well thermocycler (Applied Biosystems, USA) was used for amplification. PCR products were then separated by 2% agarose gels for 45 min at 80 V, and stained with Green viewer.

2.5. Measurement of VEGF level

Serum levels of soluble VEGF were determined using the EV 3513 cytokine biochip array (Randox Laboratories) and competitive chemiluminescence immunoassays (Randox Laboratories), according to the manufacturer's instructions, using the Randox Evidence Investigator, as described previously (Mirhafez et al., 2014).

2.6. Statistical analysis

Statistical analyses were performed using SPSS 20 (SPSS Inc., IL, USA) and Prism software (Mirhafez et al., 2015b). The normality of distribution of successive variables was decided using Kolmogorov–Smirnov test. Descriptive statistics including mean, frequency and standard deviation (SD) were determined for all variables and were expressed as mean ± SD for normally distributed variables (or as median and IQR for not normally distributed variables). For normally distributed variables, T-student test was used. The Mann–Whitney *U* test was used for continuous variables if they were not normally distributed. Chi-square or Fisher exact tests were used for categorical variables. Logistic regression analysis was used to calculate association of polymorphisms and MetS in the presence of confounders such as age and sex. The effect of SNP rs10738760 on lipid profile was analyzed using linear regression models. All the analyses were two-sided and statistical significance was set at $P < 0.05$.

3. Results

3.1. Clinical characteristics of the population

The characteristics of the subjects with and without MetS are reported in Table 1. Subject with MetS had a significantly higher BMI, waist circumference, fasting blood glucose, TC, TG, LDL, high-sensitivity CRP, SBP and DBP ($P < 0·05$) (Table 1). The HDL-C level was significantly lower in MetS group, compared to the control group (Table 1).

3.2. Association of SNP rs10738760 with MetS

In order to evaluate whether there was an association between VEGF-associated genetic-variant, rs10738760, and MetS, we carried out genotyping using DNA extracted. Genotyping was successfully performed in all the samples and the polymorphism was consistent with