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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

Table 1
Comparison of the baseline characteristics between with or without MetS.

Rs10738760		MetS − (control)	MetS+ (cases)
Frequency (N%)	Male	163 (39.7%)	168 (38.0%)
	Female	246 (60.3%)	275 (62%)*
	Total	409(100%)	443(100%)
Age (year)	Male	50.9 ± 9.1	52.7 ± 8.5
	Female	49.1 ± 8.7	52.1 ± 9.4*
Waist circumference(cm)		92.5 ± 12.4	102.1 ± 9.8*
Height (m)		160.0 ± 8.7	159.8 ± 9.5
BMI (kg/m^2)		27.9 ± 4.8	30.8 ± 4.1*
Weight(kg)		71.4 ± 13.0	78.7 ± 12.7*
Fasting blood glucose(mg/dl)		86.4 ± 25.2	97.5 ± 35.5*
Waist/weight		1.3 ± 0.2	1.3 ± 0.2
LDL-C (mg/dl)		115.6 ± 35.6	120.2 ± 38.3
HDL-C (mg/dl)		47.4 ± 10.5	39.1 ± 8.0*
Cholesterol (mg/dl)		195.2 ± 39.3	204.9 ± 45.1*
Triglyceride (mg/dl)		110 (60.8)	179(96.3)*
Hs-CRP (m/dl)		2.0 (2.7)	2.3(2.8)*
Systolic blood pressure (mmHg)		119.7 ± 16.4	133.0 ± 20.5*
Diastolic blood pressure (mmHg)		77.9 ± 9.3	85.5 ± 13.1*

MetS: syndrome metabolic, Values are expressed as mean ± SD, median and interquartile range for normally and non-normally distributed variables, respectively. 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.
* *P* value > 0.05.

the Hardy–Weinberg equilibrium ($P = 0.21$). The wild-type genotype (GG) had a frequency of 21.5%, whereas the GA and AA genotypes were found in 47.4% and 31.1% of the total population, respectively. Moreover, the wild-type rs10738760 genotype (GG) had a proportion of 23.9%, whereas the AG and AA genotypes were found in 44.5% and 31.6% of the MetS group, respectively. The corresponding frequencies in the control group were 30.6% (AA), 50.6% (AG) and 18.8% (GG). Additionally we employed logistic regression model for analysis of the association of MetS risk factors and genetic polymorphism with Mets (Tables 2–3–4). This analysis revealed that the association between MetS and its components: HDL-C, WC, TG, FBG and HTN (Table 2). Additionally we analyzed the association of rs10738760 with Mets in different genetic models, codominant, dominant, recessive and Overdominant. Interestingly we found a significant association between rs10738760 and Mets in dominant model (Tables 2–3). MetS subject carrying A/G-A/A genotypes were associated with the increased risk for MetS (OR = 1.53 (95%CI: 1.01–2.31; $P = 0.045$).

3.3. Effect of rs10738760 genetic polymorphism on circulating VEGF levels and MetS components

We then assessed the association between VEGF level and the genetic polymorphism. VEGF levels were associated with rs10738760 (Fig. 1, Table 3). In particular Mets subjects carrying AA-AG genotypes had an increased VEGF level ($P = 0.03$; Fig. 1A, Tables 2–3). Similar results were also observed in total population (Fig. 1B). Additionally we found that MetS subjects with AA + AG genotype were significant correlated with decreased level of HDL-C, compared to control group ($P = 0.0001$) (Table 4).

Table 3
Serum VEGF level in MetS group in dominant model.

	VEGF serum level (pg/ml)			
Genotype	Control	Mets	Odds ratio(95%CI)	*P* value
GG	65.2 ± 11.5	73.3 ± 11.5	Ref. 1	0.036
AA/GA	83.8 ± 14.5	126.5 ± 23.8	10(1.2–81.8)	

Ref cat: reference category, CI: confidence interval; Logistic regression analysis was used to calculate association of polymorphism and VEGF serum level with Median of 150 pg/ml in MetS. VEGF serum level was analyzed in 122 subjects. After correction for age, sex, BMI and smoking. Serum cytokines level is expressed as pg/ml.

Table 4
Serum HDL level of genotypes in Mets and control.

Genotype	Control-HDL	MetS-HDL	*P* value
GG	46.23 ± 1.098, $n = 77$	39.91 ± 0.9173, $n = 106$	0.0001
AG	47.84 ± 0.6959, $n = 207$	38.25 ± 0.5109, $n = 196$	0.0001
AA	47.31 ± 1.068, $n = 125$	39.59 ± 0.6737, $n = 140$	0.0001
AA/AG	47.64 ± 0.5909, $n = 332$	38.81 ± 0.4104, $n = 336$	0.0001
GG/AG	47.41 ± 0.5886, $n = 284$	38.83 ± 0.4635, $n = 302$	0.0001

4. Discussion

To the best of our knowledge this is the first study demonstrating the association of a genetic variant of VEGF, rs10738760 with increased risk of MetS as well as with enhanced levels of VEGF level as well as with reduced HDL level in patients with Mets. Moreover, we observed that the level of VEGF was higher in MetS patients.

There is growing body of data showing the association of MetS with insulin resistance, diabetes, CVD and chronic kidney diseases (CKD) (Tanaka et al., 2006; Chen et al., 2007; Agarwal et al., 2012; Gu et al., 2005; Kitiyakara et al., 2007). In our previous study we also detected this relationship between MetS and its components such as HDL-C and WC with CKD (Zomorrodian et al., 2015). Moreover, Agarwal et al. reported that MetS was associated with a 2.60-fold and 1.89-fold increased risk of CKD and microalbuminuria, respectively, in US adults (Agarwal et al., 2012). On the other hand, several other studies have illustrated the association of serum levels of inflammation markers with MetS and number of its metabolic traits, ischemic heart disease, heart failure, stroke, diabetes and polycystic ovary disease (Mirhafez et al.,

Table 2
Genotype frequencies for the VEGF SNP in MetS and controls in genetic models.*

Models	SNP Rs10738760	frequencies for the VEGF SNP		Odds ratio(95%CI)	*P* value
		Control	MetS		
Codominant	GG, No. (%)	68 (28.9%)	65 (21.8%)	Ref. 1	0.1
	AG, No. (%)	109 (46.4%)	145 (48.7%)	1.44 (0.93–2.24)	
	AA, No. (%)	58 (24.7%)	88 (29.5%)	1.69 (1.03–2.78)	
Dominant	GG, No. (%)	68 (28.9%)	65 (21.8%)	Ref. 1	0.045
	AA/GA, No. (%)	167 (71.1%)	233 (78.2%)	1.53 (1.01–2.31)	
Recessive	GG/AG, No. (%)	177(75.3%)	210 (70.5%)	Ref. 1	0.17
	AA, No. (%)	58 (24.7%)	88 (29.5%)	1.33 (0.89–2.00)	
Overdominant	GG/AA, No. (%)	126 (53.6%)	153 (51.3%)	Ref. 1	0.61
	AG, No. (%)	109 (46.4%)	145 (48.7%)	1.10 (0.77–1.57)	
Allele	G, No. (%)	457 (0.56)	477 (0.54)	Ref. 1	0.400
	A, No. (%)	361 (0.44)	409 (0.46)	1.06 (0.87–1.29)	

Ref cat: reference category, CI: confidence interval.
Logistic regression analysis was used to calculate association of polymorphism and metabolic syndrome.
* After correction for age, sex, BMI and smoking.

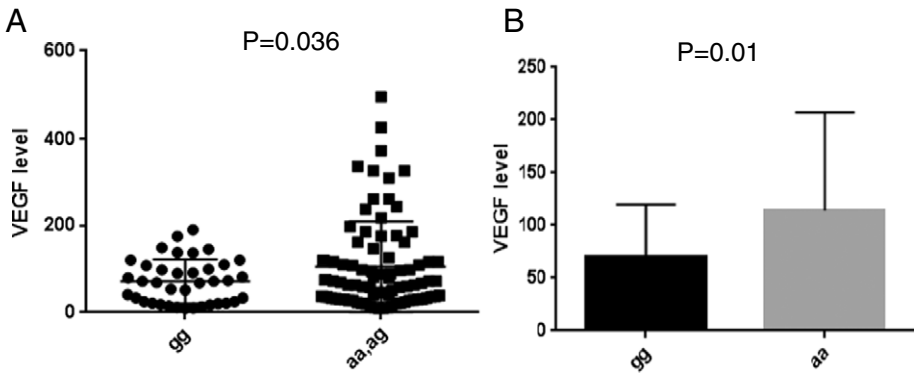

Fig. 1. Association of the VEGF genetic polymorphism with VEGF level in the MetS (A) and total population (B). Serum cytokines level is expressed as pg/ml.

2015a; Debette et al., 2011; Mirhafez et al., 2015c; Girman et al., 2004; Sutherland et al., 2004). Several studies have shown the enhanced level of VEGF in adipose tissue and its important value in response to the inflammation (Sutherland et al., 2004; Wada et al., 2010; Lieb et al., 2009b; Elias et al., 2012; Stumpf et al., 2009; Kubisz et al., 2010). Tarantino et al. reported the higher circulating level of VEGF in patients with MetS (Tarantino et al., 2007) and its role in hyperlipidemia and diabetes (Blann et al., 2001a; Ersoy et al., 2008; Kristensen et al., 2009; Barylski et al., 2009; Ayerden Ebinc et al., 2008; Petrovic et al., 2007; Miyazawa-Hoshimoto et al., 2003; Blann et al., 2001b; Belgore et al., 2000; Bonnefond et al., 2013). Pathway analyses showed a biological effect between VEGF and lipid pathways, via VLDL-triglyceride metabolism (McIlroy et al., 1999). It has been reported that the VEGF physiologic roles (such as vessel permeability) is modulated via PI3K/AKT pathway (Ferrara, 2004) and then repressed Sema3A-induced apoptosis. The VEGF is produced by foam cells and macrophages that may exacerbate atherosclerosis process through increasing vessel permeability to LDL. Moreover VEGF in hematopoietic progenitor cells inhibits nuclear factor kappa B (NFκB) pathway. In turn low physiological quantity of VEGF is needed for blood vascular homeostasis, endothelial cell survival, production of nitric oxide and prostacyclin, resulting in vasodilatation, antithrombosis and suppression of smooth muscle cells proliferation (Yla-Herttuala et al., 2007; Ramos et al., 1998; Hattori et al., 2001; Dikov et al., 2001). Several mechanisms have been proposed to change the level of VEGF, including genetic polymorphisms. Several genome wide association studies have identified an association between MetS and genetic polymorphisms in VEGF (Debette et al., 2011; Blann et al., 2001a; Kimura et al., 2007; Kraja et al., 2011b; Kristiansson et al., 2012b; Zabaneh and Balding, 2010b). In particular Debette and coworkers showed the influence of rs10738760 on serum VEGF level (e.g., A allele of rs10738760 lead to increase circulating VEGF level ($P = 1.96 \times 10^{-34}$)) (Debette et al., 2011). Also Kraja et al. reported the key role rs10738760 genetic variant with MetS (Kraja et al., 2011a). In our recent studies we revealed that rs10738760 modulated ~5% of the variation of circulating level of VEGF (Debette et al., 2011; McIlroy et al., 1999). Additionally in our previous research we found a correlation between rs10738760 and VEGF in a population from France (Blann et al., 2001a). Our recent analysis also showed that this emerging marker was associated with MetS, and MetS subjects carrying the AA/AG genotype had an increased level of serum VEGF levels and risk for MetS. In particular our data showed that AA/AG genotype had an increased risk of MetS with odd ratio of 1.5 (P value = 0.045). On the other hand, Bonnefond et al., investigated the association of two genetic polymorphisms, rs6921438 and rs10738760, in VEGF gene with the genetic risk of type 2 diabetes (T2D) and its microvascular complications in a multi-center setting of European populations (6920 T2D patients and 3875 normoglycemic controls). The SNP rs10738760 was not associated with T2D in the French ($P = 0.6$, OR = 0.98 (0.91;1.06)) and Danish populations ($P = 0.4$ (1.04 (0.96;1.12)). Furthermore we demonstrated that MetS patients carrying a AA and AG genotype had a significantly lower level of HDL-C (P value < 0.001). They found that the frequency of GG, AG and AA genotypes were 24.4%, 50.5% and 25.1% in the control group of French population, while these frequencies in case group were 25.1%, 48.6% and 26.3%. Similar results were also found in Danish population. In particular this polymorphism had a frequency of 23.6%, 50.5% and 26% for GG, AG and AA, respectively in the control group of Danish population. Also these frequencies in T2D group were 23.5%, 51.1%, and 25.3% for GG, AG and AA, respectively. Of note, the frequencies of the VEGF genotypes in our population were more or less similar with these populations (Bonnefond et al., 2013). A major strength of the present study is that it was carried out in a large number of samples with MetS, while the main limitation is age and gender differences between groups, although these variables were adjusted in logistic regression model. Another limitation is the cross sectional study design and VEGF serum level was measured in a small samples size. In addition, it is possible that lifestyle features and certain dietary intake have an effect on the correlation between VEGF genetic variant and MetS. Therefore, longitudinal studies are warranted to investigate this point.

In aggregate, we observed that patients carrying AA/AG genotypes was associated with the increased risk of MetS and correlated with decreased level of HDL, supporting further studies on the value of this genetic polymorphism in a larger population.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.gene.2016.10.034.

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