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RESEARCH ARTICLE

Exploring of Insertion/deletion Polymorphism in the Vascular Endothelial Growth Factor Gene Associated with Type 2 Diabetic Patients in Al-Diwaniyah City

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Abstract

Disease conditions that are related to the effects of the vascular endothelial growth factor (VEGF) are common such as nephropathy and promoting of cancer growth and metastasis. The current study was launched to explore the presence of VEGF gene-based polymorphism of the insertion/deletion (I/D) in blood samples of 25 diabetic and 25 clinically healthy people in Al-Diwaniyah City, Iraq. Using polymerase chain reaction (PCR), the results have shown the presence of these genotypes in these samples with higher significant (p<0.05) occurrence. The homozygous DD was significantly (p<0.05) affected by the sex factor. This genotypic characteristic was shown significantly (p<0.05) in females more than that in males. Moreover, this feature was significantly higher (p<0.05) when compared to the other genotypes II and I/D for each of the females or males separately. For the DD genotype, ages between 40 to 49 years of old have shown significantly (p<0.05) higher presence of this genotype than that in other ages. It has been also shown by these results that this genotype is present significantly (p<0.05) higher than that in the other genotypes when compared together in each age category. These results suggest current relationship between the VEGF I/D polymorphism occurrence and diabetes with deeper correlation with sex and age factors.

Keywords: *VEGF I/D polymorphism, diabetes, sex, age.*

Introduction

The vascular endothelial growth factor (VEGF) is an important cytokine that plays a crucial role in developing nephropathy and many conditions in people [1]. This factor is responsible for many pathophysiological processes in the body such as the development of angiogenesis and lymphangiogenesis due to its activation to the endothelial cell proliferation [2]. The VEGF also mediates the lymphangiogenesis activities and enhances the metastasis of breast cancer [3].

Moreover, it has been found that VEGF increases the ability of hepatocytes cancer to continue growing and promoting the bad prognosis of the cancer by metastasis [4]. For such scenario, treatments inhibiting VEGF activities could help in decreasing the detrimental prognosis of cancer [5]. Most recently, the use of VEGF blockers in mice treated with water pipe smoke has resulted in developing chronic obstructive pulmonary disease much earlier than that in mice not treated with these blockers [6]. The VEGF is a target component of treatments in many disease conditions that affect human body such as the treatment of retinopathy of prematurity in infants where it has been discovered that impairing the VEGF components was important to develop better treatment [7]. In the case of diabetic conditions especially type 2 diabetic patients, This factor was found to be important when is targeted in treatments to prevent further damages that is induced by the diabetic condition [8].

To check if the presence of the VEGF gene-based I/D polymorphism is important in featuring more or advanced destructions enhanced by the VEGF system, and because little information are found to prove the importance of these polymorphisms and their links to the diabetic condition, This study was intended to investigate these links in VEGF gene-based polymorphism of the insertion/deletion (I/D) in blood samples of 25 diabetic and 25 clinically healthy people in

Al-Diwaniyah City, Iraq. The results have proved the current study hypothesis.

Materials and Methods

Samples Collections

Using EDTA-treated tubes, blood samples from 25 diabetic and 25 clinically healthy patients were collected. The samples were obtained from The Center of Diabetes, The Teaching Hospital, and Al-Diwaniyah City. The samples were kept in a fridge to then be followed by DNA extraction procedure.

Extraction of Genomic DNA

Utilizing of Genomic DNA Mini Kit (Gene aid, USA), the collected blood samples were subjected to the DNA extraction step to obtain the genomic DNA. The DNA extraction was performed following the manufacturer's protocol that utilized frozen blood extraction method and included the use of proteinase K. The extracted DNA was furthered checked for quality and quantity using NanoDrop spectrophotometer. The DNA samples were kept frozen at -20 ℃ for later DNA-based analysis that employed PCR for the current study purposes.

PCR Analysis and VEGF Polymorphism Detection

The VEGF I/D polymorphism investigational detection was done using PCR that recruited a forward primer, GCTGAGAGTGGGGCTGACTAGGTA, and a reverse primer, GTTTCTGACCT GGCTATTTCCAGG. The primers were purchased from Bioneer Company, South Korea. AccuPower® PCR PreMix kit (Bioneer Company. South Korea) was employed to prepare the PCR mastermix solutions. The PCR premix tubes that contain frozen-dried pellets of Taq DNA polymerase 1U, dNTPs 250μM, Tris-HCl (pH 9.0) 10mM, KCl 30mM, $MgCl_2$ 1.5mM, stabilizer, and tracking dye were used to start the process of preparing the mastermix components following the kit work instructions.

Simply, 20μl of total volume was required to set up the solution by placing 5μl of the extracted DNA and 1.5μl of 10pmol of each of the forward and reverse primers. After that, the PCR premix volume was brought up to 20μl by adding deionized PCR water. Then, the prepared solution was briefly vortexed for mixing purposes.

The reactions were completed using a thermo cycler (Biorad, USA), and the following reaction conditions were used as initial denaturation at 94 °C for 5 min, 35 cycles of denaturation at 94 °C for 30 Sec., annealing at 58 °C for 30 Sec., extension at 72 °C for 30 Sec., and final extension at 72 °C for 5 min. Using agarose gel 2% and ethidium bromide-based dying, the resulted PCR products were tested by electrophoresis and visualized under a UV illuminator. The PCR-generated images were used to identify I/D polymorphism of VEGF gene. Two prospective bands should show up the amplification of a 211bp (D allele) and/or a 229bp piece (I allele).

Statistical Analyses

The data obtained from this study were processed for viability. Chi Square was used to identify the hypothetical effects of the sex and age factors on the occurrence of the I/D polymorphisms in the VEGF gene. The null hypothesis was rejected if $p<0.05$. The software used in this study was SPSS.

Results

The results showed the amplification of a 211bp (D allele) and/or a 229bp piece (I allele) in both groups, the diabetic and healthy people, figure 1. Figure 1 is an electrophoresis image of agarose gel. The figure shows the amplifications of the VEGF gene (I/D) polymorphism in the blood samples collected from diabetic patients and the healthy people. Where M is the ladder (2000-100bp), P1 is I/D genotype, P2 and P3 are DD genotype, and P4 is II genotype in diabetic patients. C1 is I/D genotype, C2 and C3 are II genotype in healthy people. I allele and D allele are at 229bp and at 211bp of product sizes respectively. In details, Table 1 and Table 2 show the results of these amplifications in the diabetic patients and the healthy people respectively.

In the case of the sex effects on the genotypes of the studied gene in the patient group, the results showed that there were significant effects ($p<0.05$) on the VEGF gene polymorphism. The DD was significantly ($p<0.05$) affected by the sex factor. This genotypic characteristic was shown significantly ($p<0.05$) in females more than that in males. Moreover, this feature was significantly higher ($p<0.05$) when compared to the other genotypes II and I/D for each of

the females or males separately. These differences are revealed in Figure 2. There were no significant effects (p>0.05) of the sex factor on these genotypes in the control group. The effects of the age factor on the VEGF gene polymorphism were significantly pronounced (p<0.05) in the patient group. For the DD genotype, ages between 40 to 49 years of old have shown significantly (p<0.05) higher presence of this genotype than that in other ages. It has been also shown by these results that this genotype is present significantly (p<0.05) higher than the other genotypes when compared together in each age category. Figure 3 shows these effects in details. There were no significant effects (p>0.05) of the age factor on these genotypes in the control group.

Samples	No. of tested sample	Genotyping (%)		
		II (homozygous)	I/D(heterozygous)	DD (homozygous)
Patient	25	4 (16%)	5 (20%)	20 (80%)
Normal	25	8 (32%)	11 (44%)	6 (24%)

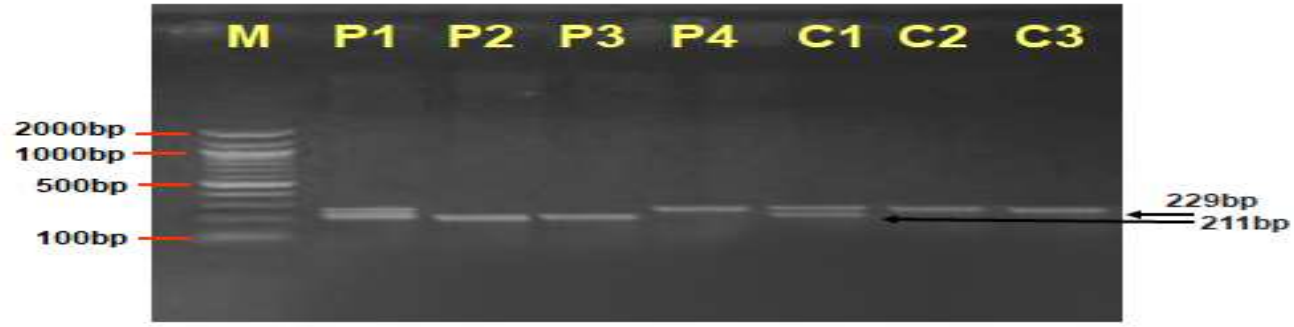

Figure 1: Electrophoresis image of agarose gel. The figure shows the amplifications of the VEGF gene (I/D) polymorphism in the blood samples collected from diabetic patients and the healthy people. Where M is the ladder (2000-100bp), P1 is I/D genotype, P2 and P3 are DD genotype, and P4 is II genotype in diabetic patients. C1 is I/D genotype, C2 and C3 are II genotype in healthy people. I allele and D allele are at 229bp and at 211bp of product sizes respectively

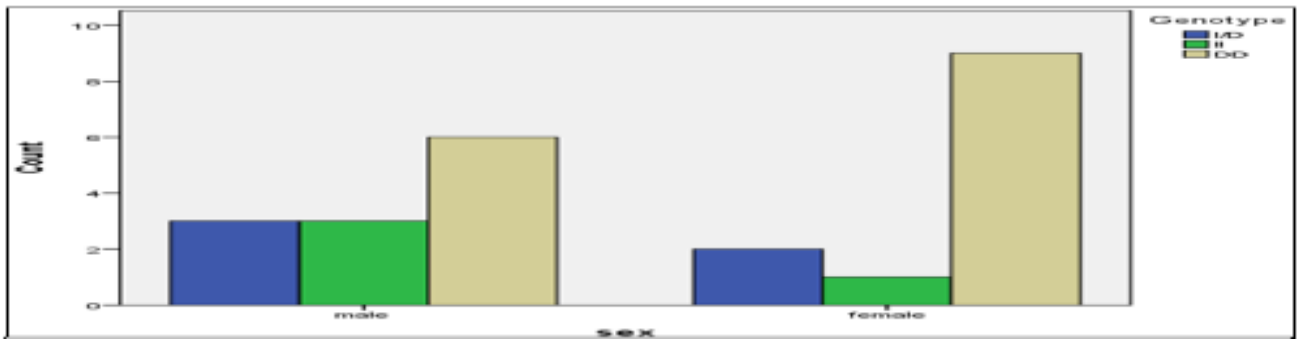

Figure 2: The effect of sex factor on the appearance of the VEGF gene polymorphism in the patient group. The DD was significantly (p<0.05) affected by the sex factor. This genotypic characteristic was shown significantly (p<0.05) in females more than males. Moreover, this feature was significantly higher (p<0.05) when compared to the other genotypes II and I/D for each of the females or males separately

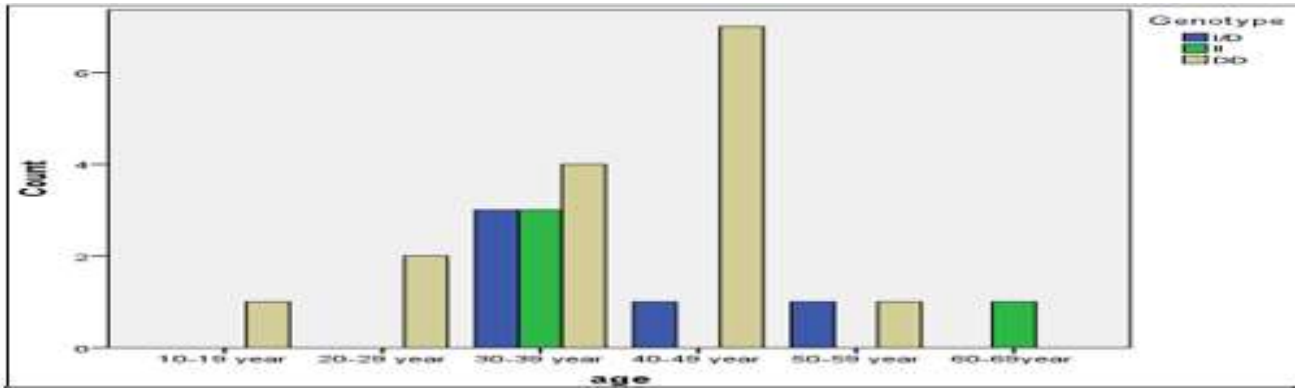

Figure 3: The effects of age factor on the appearance of the VEGF gene polymorphism in the patient group. For the DD genotype, ages between 40 to 49 years of old have shown significantly (p<0.05) higher presence of this genotype than that in other ages. It has been also shown by these results that this genotype is present significantly (p<0.05) higher than the other genotypes when compared together in each age category

Discussion

The current study results have shown the presence of the VEGF gene-related I/D polymorphisms in diabetic patients. This gives the evidence that there is an active link between type 2 diabetes and the VEGF gene polymorphisms and also presents a valued alarm of the further damages that VEGF could add in diabetic patients [9–13] that found that the VEGF polymorphism was positively correlated with the development of retinopathy.

These gene polymorphisms were significantly pronounced in the diabetic patients especially the genotype DD that showed significant correlation with sex factor. In the current investigational study, females have revealed more occurrence of the DD genotype of the VEGF gene in their samples, and this is supported by the fact that VEGF polymorphisms effects appeared in many female genital system conditions [14,16] who discovered positive correlations between these polymorphisms and the occurrence of uterine leiomyoma or polycystic ovary syndrome respectively. The physiological characteristics of females play important roles for females to be targeted by these genetic variations in the VEGF gene [17] that suggested in their study an effective role of this factor in the development of the endometriosis in rats. The DD genotypic feature could be frequently occurred in females than that in males, and this gives evidence that females might develop this polymorphism in the VEGF gene more than that in males [18] who found that some specific VEGF polymorphisms could be linked to breast cancer in females.

The DD genotypic feature was significantly ($p<0.05$) higher presence in ages between 40 to 49 years of old more than that in various ages. This indicates that these ages might have developed such a genotypic polymorphism in the VEGF gene, and this could also link and make this genotype as specific trait for these ages.

The age result could indicate that the VEGF might play a detrimental role in various parts of the body in the diabetic patients [19]. The fact that DD was the most detected genotype is an alarm of the risky situations that diabetic patients might have. The current study suggests that there are links between sex and ages of patients and the occurrence of the VEGF gene-based polymorphisms.

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