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ASSOCIATION STUDY OF VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) INS/DEL GENE POLYMORPHISM WITH RHEUMATOID ARTHRITIS PATIENTS IN BABYLON PROVINCE

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ABSTRACT : Rheumatoid is one of the common complicated diseases associated with multiple influenced factors, immunological serological and geneticplay a great role in susceptibility and diagnosis of thisdisease. *VEGF* genotyping were reported to have an influencing in rheumatoid. The present study was led to investigate the association of vascular endothelial growth factor genotypes in rheumatoid arthritis. Case-control study enrolled 100 blood samples collected from the patient attended to the Morjan Medical city, Hilla from August 2019 to March 2020. *VEGF* genotyping was performing for 50 patient's rheumatoid arthritis and 50 healthy controls using the PCR-SSP method. The diagnosis of RA was set up according to the American Rheumatism Association criteria. RA was significantly associated with VEGF. The appearance Heterogeneity alleles in RA more than control. *VEGF* genotypes have related to rheumatoid arthritis so it can be dependent as a genetic marker for the susceptibility of this disease in Iraq.

Key words : Rheumatoid arthritis, genotype, vascular endothelial growth factor.

INTRODUCTION

Rheumatoid arthritis (RA) is the more common inflammatory polyarticular. The major part of the evidence, obtained from genetics (Firestein *et al*, 2017). Rheumatoid arthritis (RA) is a chronic, symmetric, multi-joint disease that first affects the small joints of the hands and feet. The inflammatory operation is distinguished by the infiltration of inflammatory cells intothe joints (Tak *et al*, 2011; Klarenbeek *et al*, 2010). These articular and systemic Appearances are mediated by the synovium endothelial cell enlargement and extensive permeation of macrophages, lymphocytes, fibroblasts and leukocytes in the joints, wherein a variety of cytokines, prostaglandins and proteolytic enzymes are responsible for the inflammatory operation (Rommel *et al*, 2007). It is that it affects primarily the lining of the synovial joints and is associated with gradually disability, premature death and socio-economic burdens (Guo *et al*, 2018), that genetic and environmental factors are involved with the pathogenesis mechanisms of rheumatoid arthritis (RA) (Arend *et al*, 2012). Genetic factors play very important roles inthe pathogenesis ofrheumatoid arthritis. Thus, approximately 60% of the disease susceptibility genes affecting the clinical indication of RA are associated with the presence of specific alleles and the polymorphism of alleles. Vascular endothelial growth factor (VEGF), also known as vascular permeability factor (VPF), is an angiogenic factor produced by fibroblast-like synovial cells (FLS), which is stimulate angiogenesis, an invariable step in the swollen synovial tissue (pannu), VEGF also increases vascular permeability a Swelling, which leads to the accumulation of synovial fluid in the influenced joint (Mahmoodi *et al*, 2019). VEGFand its receptors are characterized in regulate (RA) by angiogenesis, VEGF work as a direct mediator of inflammation through the pathogenesis of RA (Yoo *et al*, 2008), and multiple polymorphisms within the *VEGF* gene have been associated with VEGF protein produce and have been reported to share susceptibility to numerous disorders in which angiogenesis may be critical indisease progression. Hypoxia-associated VEGF expression is attributed to increases in both transcriptional and post-transcriptional mechanisms (Han *et al*, 2004), the *VEGF* gene, genetic differences in the *VEGF* gene could have an effect on the levels of VEGF protein expression (Amle *et al*, 2015).

Activating of the VEGF-receptor pathway stimulates a network of signaling processes that support endothelial cell growth, migration and survival from pre-existing vasculature, an important role for VEGF has appeared in mobilization of endothelial precursor cells from the bone

marrow to todistantsites of neovascularization (Hicklin *et al*, 2005).

MATERIALS AND METHODS

Patients and controls

A 50 blood samples had been collect from clinically diagnosed rheumatoid patients who regularly admitted by medical committee specialized of Morjan Medical city (Babylon from August 2019 to March 2020). The patients' age range was from 25 to 60 years old include 1 patient were <20 years old, in the distribution of RA patients according to gender, 14.06% of the patients were males while 85.94% were females. In addition to 50 samples were taken from apparently healthy human were taken from Babylon province as control.

The study was accepted by the Research Ethics Review Boards of the University of Babylon. All participants provided written by formal consent case determination. Patients with rheumatoid arthritis were diagnosed by a physician based on the following criteria: These criteria were identified according to the recommendations of the American Rheumatism Association.

Blood sampling

For each individual recorded in the study, 3 ml of venous blood was collected in EDTA- tubes for DNA extraction, which performed according to the protocols recommended by the manufacturer (promega, USA).

VEGF genotype analysis

VEGF genotyping was performed with PCR-sequence-specific primers (PCR-SSP). In a 25 µl mixture of 4 µl DNA, 1.5 µl from each forward and reverse of the primer, 12.5 µl master mix and 5.5 µl nuclease-free water. The DNA amplification for *VEGF* includes an initial denaturation of 1 min in 95°C, 35 cycles of amplification (every cycle consists of denaturation of 15 s in 95°C, annealing of primers during 15 s in 61°C, and an extension of 30 s in 72°C) and a final extension of 7 min in 72°C (Amle *et al*, 2015), then the PCR products were separated in 3% agarose electrophoresis system using ethidium bromide then visualized with the gel documentation, with 100 bp-ladder and photographed. The sequences of primers used for the amplification of the genes are presented in Table 1.

Statistical analysis

All data were statistically analyzed according to software program version20 SPSS statistical software (version 17; SPSS, Inc., Chicago, IL, USA). The association between patient andcontrol and each identified *VEGF* allele and genotype was assessed using the odds ratio with its 95% confidence interval (OR, CI percentage).

RESULTS

For (*VEGF*) genotyping, the genomic DNA was amplified using specific primers and accomplished by the Thermo-cycler apparatus under the optimal conditions. The PCR products for insertion and deletion alleles *VEGF* (-2549) comprised 211bp and 229 bp, respectively.

Result of this study revealed that the VEGF genotyping had a significant variation between alleles of the RA patient and control the Ins/Ins was 18% in control, whereas it was 12% in patients, I/D genotype was more recurrent in patients (25%) than control (22%). Del/Del genotype was more recurrent in patients (13%) than control (10%) as shown in Table 2 and Fig. 1.

In this study, the SNP analysis of the VEGF gene at position -2549 INS/DEL appeared a strongly association with sensitivity of RA, that pointed out the VEGF.

Polymorphisms may be participate in the RA pathogenicity, due to it leads to change serum level of VEGF protein and RA patients (Paradowska-Gorycka *et al*, 2016).

Statistical results of the present work showed that significant variances were evident between RA cases and healthy people in terms of the allelic and genotypic distributions of VEGF V "-2549 INS/DEL" polymorphisms. We find significantly increased of ID Genotype in RA (25%) group Vs CONTROL (22%) group ($p = 0.011$).

It was concluded that the studied population of all the (100) subjects, (50) patients with RA as the study group and (50) healthy controls were genotyped for VEGF polymorphism. The average age of patients was (15-65) years. The results were obtained using the chi-square test. The frequencies of II, ID, and DD genotypes were12%, 25% and 13% in the RA group and 18%, 22%, and 10% in the controls. Odds ratio (OR) had been calculated between RA and control group for risk was

Table 1 : Sequences of the couples of primers used for the amplification of the *VEGF* gene.

Primers	Sequences5-3	Size(bp)	Reference
Forward	5' - GCTGAGAGTGGGGCTGACTAGGTA-3	229	Amle *et al* (2015)
Reverse	5' - GTTTCTGACCTGGCTATTTCCAGG-3	211	

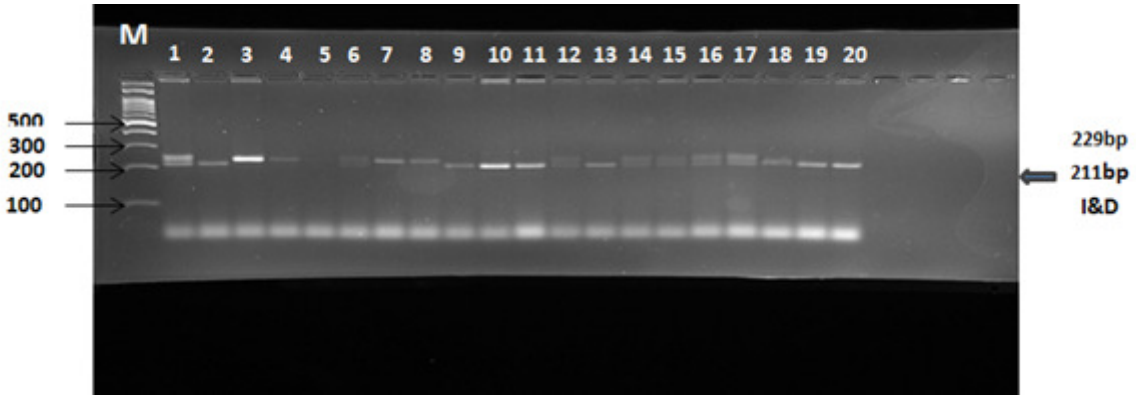

Fig. 1 : Agarose gel electrophoresis image for VEGF gene. **M:-**refers to DNA size marker line 1(100bp), line 1,6,12,14,15,16,17, *VEGF*I&D genotype, line 2,910,11,13,18,19,20 give D genotype, line 3,4,7,8, give I genotype. Electrophoresis coditions: 3% agarose concentration,100 V for 15 min, 75 V for 40 min, stained with ethidium bromide.

Table 2 : Genotype distribution and odd ratio of *VEGF* (-2549) gene polymorphism between patient and control groups.

Gene Polymorphism			Patients	Control	Total	Odd Ratio	P. Value
VEGF	Insertion	Count		18	30	2.750(1.258 – 6.010)	0.017
		% of Total	12 %	18%	30 %		
	Heterogeneity	Count	25	22	47	2.340(1.358 – 4.967)	0.011
		% of Total	25 %	22 %	47 %		
	Deletion	Count	13	10	23	2.374(1.158 – 5.322)	0.043
		% of Total	13%	10%	23%		
Total		Count	50	50	100	1.987(1.158 – 3.222)	0.002
		% of Total	50 %	50 %	100.0%		

conferred by ID genotype when was compare combined frequency of II and DD genotype, Odds of evolving RA were found to be 2.340 (1.358–4.967).

In RA patients with ID genotype when compared to RA population with II and DD genotype together.

Similar to our results, some studies have found a higher frequency of heterogeneity, distribution and allele frequencies were significantly different between RA and controls product of proinflammatory cytokinesis well as to form novel blood vessels, implying the immune response and angiogenesis in the pathogenesis of RA.

CONCLUSION

Our study provides evidence that VEGF genetic variances may be a genetic susceptibility factor for RA and that VEGF production serum levels increased in RA patients with higher disease effect. High VEGF locution may lead to the abnormal stimulation of T cells, macrophages and endothelial.

Ethical clearance

This study was approved by the Research Ethics Review Boards of the University of Babylon. All participants provided written informed consent.

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