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Association of Vascular Endothelial Growth Factor +405 Polymorphism and Psoriasis

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Abstract : Psoriasis is an inflammatory and proliferative disorder of the skin, in which both genetic and environmental influences have a critical role in pathogenesis of disease. Present study aims to assess the association of **Vascular Endothelial Growth Factor +405**genes polymorphism (SNPs) with psoriasisby PCR-RFLP while there serum levels determined by ELISA.

Methodsblood sample were collected from (64) patients with psoriasis (40 males and 24 females) and (38) healthy people as control group matched with disease group. samples collected in Marjan Medical City in Babylon Province. Polymorphism (SNPs) determined by Polymerase Chain Reaction (PCR), restriction fragment length polymorphism (PCR-RFLP) and DNA sequencing techniques, while there serum levels determined by ELISA.

Results: The results show significant increasing in VEGF level in patient compare with controls ($P<0.01$) while There is no correlation between VEGF serum level and severity or onest of disease (-r=-0.05). The genetic analysis of the Single Nucleotide Polymorphisms of VEGF +405 SNP, genotype distribution were CG heterozygous was predominant in patient 33(60.94%) and GG homozygous was 24 (37.05) while the homozygous CC was the lest 1 (1.56%). Comparing with control group CC 13(34.21%) GG 7(18.42%) and CG 18(47.37%).

Conclusions: the study confirmed an association between the increase of VEGF serum level and susceptibility to psoriasis and the massive majority of single nucleotide polymorphism in *VEGF* +405 genes have a clinical importance concerning psoriasis.

Key word : psoriasis, Vascular Endothelial Growth Factor polymorphism, VEGF-RFLP.

Introduction

Psoriasis is a common, chronic, and immune-mediated inflammatory skin disease that affects individuals at proportions varying from 0.5% to 4.6% across various ethnic populations[1].Psoriasis is characterized by clinical appearance of red, heavily scaled skin plaques with dense infiltrates of T cells, macrophages, and dendritic cells (DC) as well as hyper-proliferation and inadequate differentiation of epidermal keratinocytes[2].

The vascular network found within these lesions is highly altered, especially in the papillary dermis, which infiltrated, by a large number of tortuous and dilated capillaries. Some Mutations promote the expansion of the vascular network through the secretion of pro-angiogenic factors such as VEGF and angiopoietins, which exert pro-angiogenic action as well as activation of endothelial cells. Vascular endothelial growth factor (VEGF) recognized as a main epidermal-derived vessel-specific growth factor that intensely up regulated in

psoriatic skin lesions combined with modification in VEGF gene [3]. Thus, VEGF may play an important causative effect in mechanisms contributing the development of psoriasis[4].

Genetic susceptibility factors affecting both the immune system and epidermis could predispose to disease[5].several genetic studies promote the associated of several VEGF SNPs with early-onset psoriasis [−2578(C/A),+405(C/G) and −460(C/T)] [6]. Young *et al (*2006) found that the production of VEGF by peripheral blood mononuclear cells influenced by the genetic polymorphism whereas secretion by keratinocytes did not[7].

This study aims to assessVEGF genes polymorphism associated with psoriasis patientsusing Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) andevaluate therelationships between VEGF and clinical parameters of disease.

Materials and methods

Study Subjects:

64 Iraqi patients with psoriasis selected randomly from patients admitted to the dermatology department and 38 healthcontrol subjectsall examined in this study.

Genotyping:

Genomic DNA were extracted from peripheral frozen whole blood according to protocol of DNA extraction kit (fovergen/ korea). VEGF + 405genetic polymorphisms analyzed by (PCR-RFLP) With primers: forward F: 5'-ATTTATTTTTGCTTGCCATT-3'and revers R: 5'-GTCTGTCTGTCTGTCCGTCA-3'. PCR achieved at 94^{O}C for 2min followed by 35 cycle of 94^{O}C for 4 min, annealing at51^{O}C for 30min extension at 72^{O}C for 30min and final extension at 72^{O}C for 10min . PCR products 304bp were digested by restriction enzyme *BsmFI* to produce tow fragment 111bp and 193bp, visualized by electrophoresis on 1.5% agarose gel stained with ethidium bromide[8].

VEGF serum level:

serum level of VEGF were determined by ELISA according to to manufacturing protocol of ELISA kit (Elabsit/ chaina).

Statistical analysis:

Statistical analysis carried out using SPSS version 23. Continuous variables as a mean ± SE for serum VEGF level and genotyping frequency tested by Hardy–Weinberg equilibrium.

Results and Discussions

A Case control study samplesinclude 64 patient with psoriasis and 38 healthy control. The majority of patients were males (62.5%) while (37.5 %) were females. (43.75 %) of them were smokers, the other were not. the present study it is found that 31(49.44%) of psoriasis patients had a positive family history, while 33 (51.56%) of those who have a sporadic cases of psoriasis.

Table (1). Distribution of study samples according to gender, smoking andPositive family association

variable	Control (%)	Patients (%)	X^2	p-value
Gender :				
Male	21 (55.24%)	40 (62.50%)		
Female	17 (44.74%)	24 (37.50%)	3.052	p>0.5
Smoking				
Smoker	9 (23.68%)	28 (43.75%)		
Non-smoker	29 (76.32%)	36 (56.25%)	2.082	p>0.5
severity				

Mild to moderate (PASI<20)	--	33(51.563%)		
Sever (PASI≥20)	--	31(48.437%)	1.53	p>0.5
Familial association				
sporadic		33(51.6%)		
familial		31(48.4%)		
type				
Type 1		50(78.13%)		
Type 2		14(21.87%)		p<0.5
Total	38	64		

There are not significant association between patients with positive family association and those with sporadic, the control group included 38 healthy volunteers, 17 males (44.74%) and 21 females (55.24%). Their age ranged between18 and 70 years. Controls were age and sex matched.Table (1) show the distribution of study samples.

Psoriasis affects about 125 million of people worldwide. It is common in Caucasians and affects correspondingly men and women [9].Psoriasis also affects about 2-3% of the population and it was equally distributed between the both sex males and females[10]. psoriasis patients comprised36 (51.4%) females and 34 (48.6%) males and the ratio of females to males was (1:1)[11].

The PASI score for clinical assessment ranged between 6.8 and 58.7. Thirty-three of the patients had mild to moderate psoriasis (PASI < 20), while the other 33 had severe psoriasis (PASI ≥20). Present result covenant with karam*et al* (2014) whose found the PASI range (7-65) , (35.5%) of the patients had mild to moderate psoriasis, while the other (64.5%) had severe psoriasis[12]. Severity of disease and symptoms werereflect the heterogeneity, risk factor exposure and treatment[13].

As show in table (1), the patients were31(48.4%) considered familial whose had one or more first- to third-degree relatives psoriasis condition and 33(51.6%) sporadic patients. The positive family history of psoriasis varied from (30 % to 60 %) in European studies and from (10% to 30%) in studies in other countries[14]. Chiriac*et al*. (2014) demonstrated an association of family history of (29.53%) with psoriasis patients[15]. Na *et al*. (2013) found an association of family history of (26%) with psoriasis patients[16].Bahcetepe*et al*. (2013) found that (56%) of cases had positive family history[17].

VEFF serum level:

serum concentration of VEGF were measured in 64 psoriasis patients and 38 control,Mean VEGF plasma levels were (346.22 ± 25.53 pg/ml) in patients and (225.47 ±23.31pg/ml) in controls, respectively. This difference between groups was statistically significant (P<0.0018).

A significantly increased serum levels of VEGF in patients with psoriasis compared with healthy controls (P =0.0018). Mean VEGF serum levels were (346.22 ± 25.53pg⁄mL) in patients and 247.87 ± 151.26 pg⁄mL (range 42.2–553.0 pg⁄ mL) in controls, respectively.

The result coms agreed with Yalçin*et al.*, 2007 which find that the VEGF serum levels are increased in psoriatic patient compared with normal[18]. The mechanisms responsible for VEGF level altrationare complex and involve secretion by keratinocytes, leukocytes and possibly even neurons[19].

Table (2) mean difference of Serum level of VEGFbetween Psoriasis Patients and controls.

Biomarker / Groups	(Mean ± SE) pg/ml
	VEGF
Patients	346.22 ± 25.53
Control	225.47 ±23.31
T-Test	74.838 **
P-value	0.0018
** (P<0.01).	

Genetic studies have associated several VEGF SNPs to early-onset psoriasis [−2578(C/A), −460(C/T) and +405(C/G)][20]. Young *et al (2006)*. found that the production of VEGF by peripheral blood mononuclear cells depended on the genotype, whereas production by keratinocytes did not[7].

Aside from genetic predispositions, many factors found in high concentration within scales, such as epidermal growth factor (EGF), transforming growth factor- β1 (TGF-β1) and tumor necrosis factor- α (TNF-α), contribute to the high concentration of VEGF by promoting its secretion[21]. Up regulation of VEGF by pro-inflammatory cytokines could explain the expansion of the vascular network following the injection of activated immunocytes within the skin of xenotransplantation animal model[22].

VEGF +405 genotyping

Genotyping ofVEGF-A +405 was carried out by polymerase chain reaction (PCR), Positive amplification results a PCR products (304bp) (Bozduman *etal*, 2016).were separated on 2% agarose stained with ethidium bromide and translucenceby ultraviolet light.

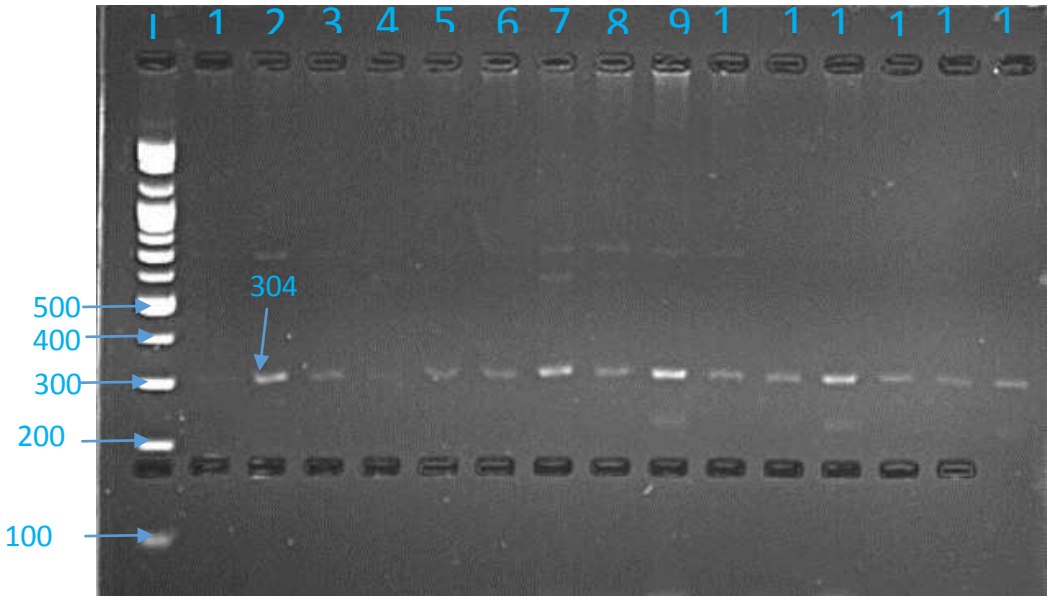

Figure (1) Gel electrophoresis for VEGF-A +405gene PCR products Separated on a 1.5% agarose gel stained with ethidium bromide, 75V, 20Am for 2 h. L: 100 bp marker; lane 1-10: Psoriasis patients lane11-15 controls.

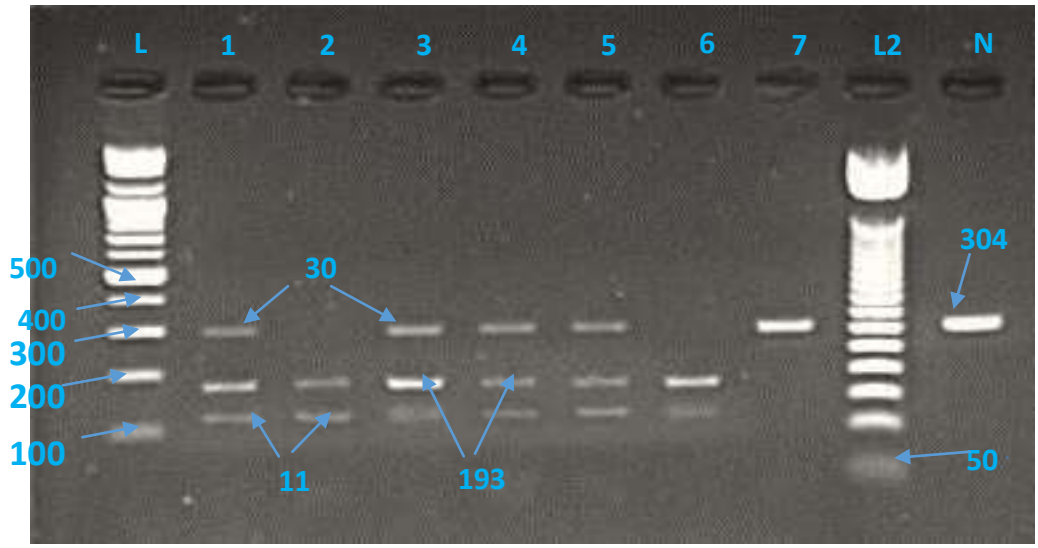

Figure (4-8) Gel electrophoresis for VEGF-A +405 gene PCR products digested with BsmFI restriction enzyme. Separated on a 3.5% agarose gel stained with ethidium bromide 75V, 20Am for 2 h.. L: 100 bp marker, lane 1-4 psoriasis patient, lane 5-7 control , L2 50bp marker and lane N undigested product.

An significant difference of Number percentage distributions of alleles and genotypes for this locus between patient and control . As summarized in Table (3) CG heterozygous was predominant in patient 33(60.94%) and GG homozygous was 24 (37.05) while the homozygous CC was the lest 1 (1.56%). Comparing with control group CC 13(34.21%) GG 7(18.42%) and CG 18(47.37%).

Table (3) Number and percentage of Genotype and allele frequency in patients and control of VEGF +405

Genotype (VEGF gene)	Control No. (%)	Patients No. (%)	O.R.	CI
CC	13 (34.21%)	39 (60.94%)	1.188	0.89-1.62
CG	18 (47.37%)	1 (%1.56)	1.542	0.91-1.63
GG	7 (18.42%)	24 (37.05%)	0.894	0.88-1.63
Total	(100%%)	64 (100%)	---	---
Chi-square (χ^2)	9.026 **	12.835 **	---	---
Allele frequency				
C	0.58	0.62		
G	0.42	0.38		
** (P<0.01).				

The VEGF-A genotype frequencies in the study groups are shown in Table (3). +405 SNP tested were in Hardy–Weinberg equilibrium in cases and controls (P > 0.01). Allele frequencies were significantly different between patients with psoriasis and controls. The +405G/C locus has been found to be associatedwith a great number of inflammatory or neoplastic diseases as well as with VEGF production[23].

+405 CC SNP in VEGF gene associated and significantly increased in patient with Type I psoriasissupport the role of VEGF gene polymorphism in the pathogenesis of psoriasis[24].

Our study finding coms similar to Young *et al*(2004) showed that the +405CC genotype confers a risk of developing severe psoriasis and a disease onset between the ages of 20 and 40 years.The -460C/+405G haplotype was found to be the most commonly observed haplotype in the normal population[20].

An ethnic/racial contribution to the distribution of common VEGFA variants, and significant associations between VEGFA genotype and both VEGF secretion and disease states have been reported[25].

Table 4show the relation between serum level and the genotypes of VEGF in psoriatic patients, the deference non-significant between homozygous CC (408.30 ± 0.00) , homozygous GG (326.00 ± 34.81) and heterozygous CG (376.41 ± 58.63). Many incompatible results have been published on the relationship between VEGF sequence variations and the amount of protein produced [26,20]

Table (4) serum level of VEGF in relation to their genotypes

Genotype of VEGF gene	No.	Mean ± SE of VEGF level
CC	39	408.30 ± 0.00
CG	1	376.41 ± 58.63
GG	24	326.00 ± 34.81
LSD value	---	102.537 NS
P-value	---	0.6022
NS: Non-significant		

The +405G/C locus has been found to be associatedwith a great number of inflammatory or neoplastic diseases as well as with VEGF production. Some authors reported that high VEGF production was associated with +405CC [27].while others indicated that high VEGF serum level is associated with +405GG genotype[26,28].

References

1. Lebwohl, M., Tyring, S. K., Hamilton, T. K., Toth, D., Glazer, S., Tawfik, N. H., ...&Pariser, D. (2003). A novel targeted T-cell modulator, efalizumab, for plaque psoriasis. *New England Journal of Medicine*, *349*(21), 2004-2013.
2. Griffiths, C. E., & Barker, J. N. (2007). Pathogenesis and clinical features of psoriasis. *The Lancet*, *370*(9583), 263-271.
3. Detmar, M. (2004). Evidence for vascular endothelial growth factor (VEGF) as a modifier gene in psoriasis. Journal of investigative dermatology, 122(1), xiv-xv.
4. Xia, Y. P., Li, B., Hylton, D., Detmar, M., Yancopoulos, G. D., &Rudge, J. S. (2003). Transgenic delivery of VEGF to mouse skin leads to an inflammatory condition resembling human psoriasis. *Blood*, *102*(1), 161-168.
5. Sun, L., & Zhang, X. (2014, December). The immunological and genetic aspects in psoriasis. In *Applied Informatics* Vol. 1, No. 1, p. 3. Springer Berlin Heidelberg.
6. Wongpiyabovorn, J., Yooyongsatit, S., Ruchusatsawat, K., Avihingsanon, Y., &Hirankarn, N. (2008). Association of the CTG (− 2578/− 460/+ 405) haplotype within the vascular endothelial growth factor gene with early-onset psoriasis. *Tissue antigens*, *72*(5), 458-463.
7. Young, H. S., Summers, A. M., Read, I. R., Fairhurst, D. A., Plant, D. J., Campalani, E., ...& Griffiths, C. E. (2006). Interaction between genetic control of vascular endothelial growth factor production and retinoid responsiveness in psoriasis. Journal of investigative dermatology, 126(2), 453-459.
8. Bozduman, T., Ersoy Evans, S., Karahan, S., Hayran, Y., Akbiyik, F., & Lay, I. (2016). Genetic Risk Factors for Psoriasis in Turkish Population:-1540 C/A,-1512 Ins18, and+ 405 C/G Polymorphisms within the Vascular Endothelial Growth Factor Gene. *Annals of dermatology*, *28*(1), 30-39.
9. Langley, R.G.B.; Krueger, G.G. and Griffiths, C.E.M. (2005). Psoriasis: Epidemiology, clinical features, and quality of life. Annu. Rheumat.Dise.; 64(2): 18 –23.
10. Duarte, G. V.; Follador, I.; Cavalheiro, C. M. A.; Silva, T. S. and De Oliveira, M. D. S. P. (2010). Psoriasis and obesity: literature review and recommendations for management. An. Bras. Dermatol.; 85(3): 355-360.
11. Abbas, E. C. (2013). The role of Staphylococcus aureussuperantigen in the pathogenesis of psoriasis vulgaris. P.H.D. Thesis. Collage of Medicine, Kufa University, Iraq. 123-153.
12. Karam, R. A., Zidan, H. E., &Khater, M. H. (2014). Polymorphisms in the TNF-α and IL-10 gene promoters and risk of psoriasis and correlation with disease severity. Cytokine, 66(2), 101-105.
13. Barile, S., Medda, E., Nistico, L., Bordignon, V., Cordiali-Fei, P., Carducci, M., &Bonifati, C. (2006). Vascular endothelial growth factor gene polymorphisms increase the risk to develop psoriasis. *Experimental dermatology*, *15*(5), 368-376.

14. Zhang, X. J.; Wang, H. Y.;Te-Shao, H.; Yang, S. andChen, S. (2002).The genetic epidemiology of psoriasis vulgaris in Chinese Han. Int. J. Dermatol.; 41 (10): 663-669,
15. Chiriac, A.; Solovan, C.; Chiriac, E. A.; Foia, L. and Brzezinski, P.. (2014). A case-control study of epidemiological importance risk of family history of psoriasis. Our. Dermatol. Onl.; 5(1): 90-91.
16. Na, S. J.; Jo, S. J. and Youn, J. I. (2013). Clinical study on psoriasis patients for past 30 years (1982-2012) in Seoul National University Hospital Psoriasis Clinic. J. Dermatol.;40 (9): 731-735.
17. Bahcetepe, N.; Kutlubay, Z.; Yilmaz, E.; Tuzun, Y. and Eren, B. (2013). The role of HLA antigens in the aetiology of psoriasis. Med. Glas. (Zenica);10 (2): 339-342.
18. Yalçin B, Tezel GG, Arda N, Erman M, Alli N (2007) Vascular endothelial growth factor,vascular endothelial growth factor receptor-3 and cyclooxygenase-2 expression in psoriasis. Anal Quant CytolHistol 29:358–364.
19. Guérard, S., &Pouliot, R. (2012). The role of angiogenesis in the pathogenesis of psoriasis: mechanisms and clinical implications. J ClinExpDermatol Res S, 2 (2).
20. Young, H. S., Bhushan, M., Griffiths, C. E., Summers, A. M., &Brenchley, P. E. (2004). Single-nucleotide polymorphisms of vascular endothelial growth factor in psoriasis of early onset. *Journal of investigative dermatology*, *122*(1), 209-215.
21. Heidenreich, R., Röcken, M., &Ghoreschi, K. (2009). Angiogenesis drives psoriasis pathogenesis. *International journal of experimental pathology*, *90*(3), 232-248.
22. Nickoloff, B. J., & Nestle, F. O. (2004). Recent insights into the immunopathogenesis of psoriasis provide new therapeutic opportunities. *The Journal of clinical investigation*, *113*(12), 1664-1675.
23. Stevens, A., Soden, J., Brenchley, P. E., Ralph, S., & Ray, D. W. (2003). Haplotype analysis of the polymorphic human vascular endothelial growth factor gene promoter. Cancer research, 63(4), 812-816.
24. Zablotna, M., Sobjanek, M., Nedoszytko, B., Lange, M., Kozicka, D., Glen, J., &Roszkiewicz, J. (2013). Association of psoriasis with the VEGF gene polymorphism in the northern Polish population. *Journal of the European Academy of Dermatology and Venereology*, *27*(3), 319-323.
25. Al-Khateeb, G. M., Mustafa, F. E., Sater, M. S., &Almawi, W. Y. (2011). Effect of the functional VEGFA− 583C/T variant on vascular endothelial growth factor levels and the risk of recurrent spontaneous miscarriage. *Fertility and sterility*, *95*(8), 2471-2473.
26. Salvarani, C., Boiardi, L., Casali, B., Olivieri, I., Cantini, F., Salvi, F., ...&Filippini, D. (2004). Vascular endothelial growth factor gene polymorphisms in Behcet's disease. The Journal of rheumatology, 31(9), 1785-1789.
27. Lambrechts, D., Storkebaum, E., Morimoto, M., Del-Favero, J., Desmet, F., Marklund, S. L., ...& Al-Chalabi, A. (2003). VEGF is a modifier of amyotrophic lateral sclerosis in mice and humans and protects motoneurons against ischemic death. Nature genetics, 34(4), 383-394.
28. Watson, C. J., Webb, N. J., Bottomley, M. J., &Brenchley, P. E. (2000). Identification of polymorphisms within the vascular endothelial growth factor (VEGF) gene: correlation with variation in VEGF protein production. Cytokine, 12(8), 1232-1235.

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