

THE EFFECT OF TNFAIP3 GENE POLYMORPHISM on DISEASE SUSCEPTIBILITY AND RESPONSE of ETANERCEPT in PSORIATIC PATIENTS

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Abstract:

Background: Psoriasis is a chronic inflammatory skin disease has a strong genetic predisposition.

Aim of the work: To study the role of *TNFAIP3* rs610604 (C/A) polymorphism in psoriasis, and its effect on Etanercept response.

Subjects and methods: One hundred patients with psoriasis, in addition 100 apparently healthy individuals as a control group were included in this study. Restriction Fragment Length Polymorphism-Polymerase Chain Reaction (RFLP-PCR) was performed to evaluate different *TNFAIP3* gene polymorphism. *TNFAIP3* serum levels were assessed by enzyme-linked immunosorbent assay.

Results: Current study showed low serum level of TNFAIP3 protein in psoriatic patients when compared with control group (P<0.01). A considerably higher risk of psoriasis was observed in individuals who had TNFAIP3 rs610604 SNP CC and C allele than people carrying AA genotype and A allele. In relation to the response to treatment, this study observed that the mutant homozygous genotype CC was more frequent in non-responder (71.4%) than responder (1.3%) patients (P<0.01).

Conclusion: *TNFAIP3* rs610604 (C/A), might be useful for prognosis of psoriatic patients.

Keywords: Psoriasis, *TNFAIP3* gene and Etanercept

INTRODUCTION

Psoriasis (Ps) is a multifactorial, chronic inflammatory disorder that has a considerable effect on health care. By mediating intercellular contact between immune cells that invade the skin and keratinocytes, cytokines have an important role in disease pathogenesis. [1]. Psoriasis prevalence varies with geographic location and has been recorded to range from 1% to 3% [2]. Family and twin studies suggest that genetic predisposition plays a significant role in the growth of Ps [3]. Moreover, multiple studies have confirmed that environmental factors are capable of causing the disease [4]. Some TNF alpha-induced protein 3 gene (TNFAIP3) polymorphisms have been associated with anti-TNF response therapy in patients with Ps. [5]. Tumor necrosis factor alpha-induced protein 3 (TNFAIP3) was originally described as a TNF inducible gene that acts as a negative TNF signaling feedback inhibitor, at least in certain settings. [6]. Its protein product, A20, acts as a dual enzyme: it initially removes the receptor-

interacting protein 1 (RIP1) chain of Lys63-linked ubiquitin, an important mediator of the proximal TNFR signaling complex. Subsequently, it functions as E3 ligase, resulting in RIP1 polyubiquitinylation via Lys48 targeting RIP1 for proteasomal degradation, resulting in TNF-induced nuclear factor kappa-light-chain-enhancer termination of activated B-cell nuclear factor kappa β (NF- κ B) signaling cells. [7]. *TNFAIP3* gene has been associated with Psoriasis, Psoriatic arthritis and Rheumatoid arthritis, as well as with other autoimmune disorders [8].

SUBJECTS and Methods

This case-control study was performed in Baghdad city from January to December 2019. This study included one hundred psoriatic patients their age ranged from 15 to 70 years. They were seeking on Etanercept treatment in the biological drug center at Baghdad teaching Hospital and Al-Imamain Al-Kadimain Medical City. Each case was diagnosed by a dermatologist. Also this study included 100 apparently healthy subjects as a control group. Inclusion criteria: One hundred psoriatic patients treated with biological drug (Etanercept) one dose (25-50 mg) per week for 6 month as average. Exclusion criteria: psoriatic patients had not treated with biological drugs. Three mls of venous blood were collected from each patients and controls; 2 mL of which were kept in the Ethylenediaminetetraacetic acid tube (EDTA tube) and the other 1ml in gel tube. The sera used to determine the level of human TNFAIP3 proteins in serum. D.GSYNCTM DNA Extraction Kit Quick / Geneaid – South Korea was used to extract the DNA. SNP genotyping was performed by the Restriction Fragment Length Polymorphism-Polymerase Chain Reaction (RFLP-PCR) according to the manufacturer's instruction. Primers were used in this study to amplify the corresponding fragments of TNFAIP3 rs610604 (C/A) gene designed based on NCBI database table (1) and thermal cycling conditions for amplification of the *TNFAIP3* gene (rs610604 C/A) show in table (2) .

Table (1): Primers and their corresponding single nucleotide polymorphisms for *TNFAIP3* gene in the present study

Polymorphisms	Primers5'→3'	Restriction enzymes	Product Size (bp)
rs610604 (C/A)	F- GGCGTTTCACATTTTACATCCA R- TAGACTAGTCCCAAACCAATGGC	SacI	334bp+244bp (C) 578 bp (A)

PCR optimal conditions were applied for the amplification of TNFAIP3 rs610604 (C/A) gene, as shown in table (2).

Table (2): Thermal cycling conditions for amplification of the *TNFAIP3* gene (rs610604 C/A)

Step	Temperature and duration	
Initial denaturation	94oC for 4 minutes	
Denaturation	94oC for 30 second	35 cycles
Annealing	55oC for 30 second	
Extension	72oC for 45 second	
Final extension	72oC for 7 minutes	

Statistical Analysis

Percentage frequencies, alleles variations were presented in term of frequency and odds ratio (OR). P value of $p < 0.05$ was considered statistically significant.

RESULTS

There were no statistical significant differences between two studied groups according to age and sex ($p > 0.05$). The mean age of patients was 37.34 ± 14.372 year and for healthy controls was 36.33 ± 13.92 year. It was found that age, sex, body mass index and disease onset

variables did not differ significantly between the Etanercept responders (79) and no responders (21) patients except for the positive family history that was higher in non-responder (85.7%) than in responder patients (24.1%) at a highly significant difference ($p=0.0001$). Regarding cigarette smokers in non-responder was higher (66.7%) than in responder patients (31.6%) with significant difference ($p=0.003$). In addition, non-responder have higher percentage of psoriatic area and severity index (PASI) (90.5%) than in responder patients (2.5%) with significant difference ($p=0.0001$). Moreover, higher Psoriatic arthritis was in non-responder (57.1%) than responder patients (6.3%) with a highly significant difference ($p=0.001$). The frequency of the AA, AC and CC genotypes of TNFAIP3 rs610604 were 18.0%, 66.0% and 16.0% respectively in patients compared to 25.0%, 75.0% and 0% respectively in controls Figure (1). There was a higher significant frequency of mutant homozygous genotype (CC) in patients compared to control group (Likelihood Ratio: 23.9, $P<0.0001$, $P= <0.0001$) table (3) and table (4) shows the frequency of different alleles of TNFAIP3 rs610604 polymorphism in patients and controls.

Table (3): The frequency of different genotypes of TNFAIP3 rs610604 polymorphism in patients and controls

Chi-square: $P<0.0001$ Likelihood Ratio: 23.9, $P<0.0001$		SNP TNFAIP3 rs610604			
		wild AA	heterozygous AC	mutant CC	Total
Patients vs controls	Patients	18 18.0%	66 66.0%	16 16.0%	100 100.0%
	Controls	25 25.0%	75 75.0%	0 0%	100 100.0%

At allele level with frequency of a mutant allele (C allele) in patients was more than control with significant difference ($OR=1.6$, $P=0.02$) table (4). This polymorphism had three genotypes (AA, AC, and CC) in patients and controls figure (1).

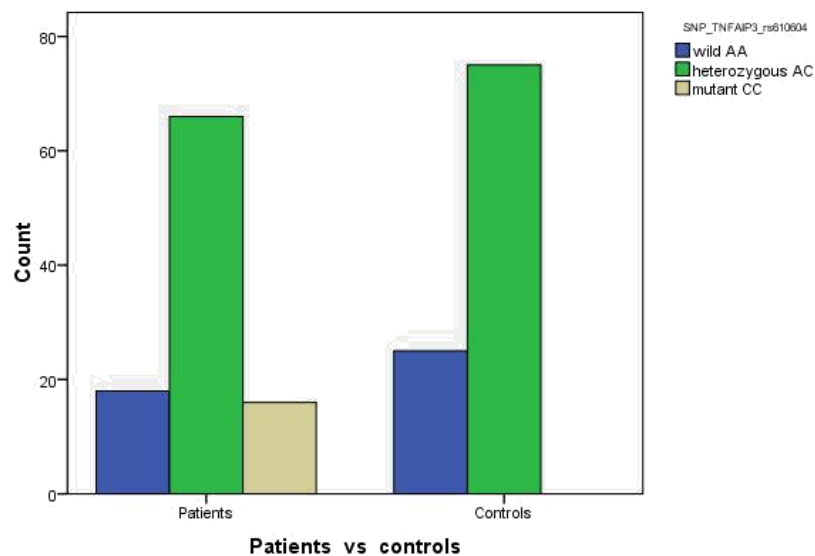


Figure (1): The frequency of different genotypes of TNFAIP3 rs610604 polymorphism in patients and controls

Table (4): The frequency of different alleles of TNFAIP3 rs610604 polymorphism in patients and controls

P=0.02		Count/%	Allele A	Allele C	Total
Odds ratio=1.6, P=0.02					
SNP_TNFAIP3_rs610604					
Patients	Count	102	98	200	
	%	51	49	100	
Controls	Count	125	75	200	
	%	62.5	37.5	100	

The mutant homozygous genotype (CC) was more frequent in non-responder (71.4%) than responder (1.3%) patients with highly significant differences in the distribution of different genotypes of this polymorphism between responder and non-responder patients table (5).

Table (5): The frequency of different genotypes of TNFAIP3 rs610604 polymorphism in responder and non-responder patients

P<0.0001		SNP TNFAIP3 rs610604			
Likelihood Ratio: 52.6, P<0.0001		wild	heterozygous	mutant	Total
		AA	AC	CC	
Response to treatment	NO	2 9.5%	4 19.0%	15 71.4%	21 100.0%
	Yes	16 20.3%	62 78.5%	1 1.3%	79 100.0%

Current study showed a high significant difference between overall patients and controls, concerning serum level of TNFAIP3 the comparison revealed level of TNFAIP3 in patients with median= 2.32 and in control with median= 8.52 ng/ml with (P=0.001). Also, there was a significant difference in serum levels of TNFAIP3 was found between responder and non-responder patients (P=0.01) figure (3).

DISCUSSION

There was non-significant difference (P=0.61) in the present study between the mean age of patients and controls, the disease affected almost all age groups. This observation is similar to Basko-Plluska JL *et al.* [9] Who discovered Psoriasis can develop at any age, but the larger number of cases, approximately 75%, occur before the age of 40, male psoriatic patients (57.0%) were seen more frequently than in females (43.0%); this finding is comparable in other previous studies. [10-12]. Some studies have shown, however, that this disease is the same or more frequent in women. [13]. This may be a due to limited access to health care facilities in case of females in our community. The genetic factors that related with psoriasis have not been fully illustrated, the human leukocyte antigens (HLA) have been considered candidate marker for psoriasis because they are contributed in regulating the immune responses [14]. Abbas AA. (15) Revealed that the frequencies of HLA- C*12, HLA- C*17, HLA- DRB1*07 and HLA-DQB1*02 were significantly higher among patients as compared to control (P=0.0174; P<0.0001; P=0.0136 and P=0.0004 respectively). On the other hand low frequencies of HLA-C*04 and HLA- DQB1*01 alleles were found in patients when compared with control (P=0.0003 and P=0.0009 respectively). In the current study, a considerably higher risk of psoriasis was observed in subjects who had TNFAIP3 rs610604 SNP CC and C allele than carrying AA genotype and A allele; this result is in agreement with Zhang C. *et al.* [16] who indicated that SNP rs610604 is associated with risk of psoriasis in Chinese population. The significance of this observation is that the clinical incidence of psoriasis in the genotype-phenotype study was correlated with this SNP. This may provide a new understanding of the etiology of complex clinical psoriasis presentations. A20, a TNF-a-inducible zinc finger protein, encodes the TNFAIP3 gene. As a negative immunoregulatory protein, A20 plays an important role in the negative feedback regulation of NF- β B (nuclear

factor kappa light-chain enhancer of activated B cells) signaling. [17]. In addition, A20 also controls TNF-induced apoptosis and acts on the NF- κ B signaling pathway at several levels. [18] A20 protein could restrict activation of T cells by directly inhibiting NF- κ B activation [19] or down regulating the T cell stimulatory capacity of dendritic cells [20]. A series of previous studies have documented SNP rs610604 in intron 3 of TNFAIP3 is associated with psoriasis in Chinese Han and white populations [21]. Zhang C. *et al.* found that SNP rs610604 C allele is associated with the risk of having psoriasis and with the clinical severity of Psoriasis as well. One possible explanation for this result is that lower A20 mRNA levels are correlated with the SNP rs610604 C allele [22]. Another research has previously reported that A20 mRNA expression in the extreme group was lower than in the moderate group and associated negatively with psoriasis disease severity [17]. This indicates that low TNFAIP3 expression could affect the function of the protein in such a way that it is not possible to limit inflammation and NF- κ B signaling, leading to severe psoriasis. The level of expression of the TNFAIP3 gene can play a critical role in psoriasis pathology and is directly involved in the psoriasis pathological process. In relation to the response to treatment, this study observed that the mutant homozygous genotype CC was more frequent in non-responder (71.4%) than responder (1.3%) patients with a highly significant difference. Trilokraj Tejasvi *et al.* [23] found good response to Etanercept alone and all TNF blockers combined was positively associated with the C allele of rs610604. The current study shows that low serum level of TNFAIP3 protein in psoriatic patients when compared with control group with a high significant difference. Also a high serum levels of TNFAIP3 in responder than non-responder patients with significant difference; this finding is similar to Nahla Yassin Sahlol *et al.* [24] who demonstrated that the expression of TNFAIP3 gene is altered in psoriasis, as evidenced by a significant down-regulated expression of TNFAIP3 mRNA in blood and skin of psoriatic patients compared to controls and a significant decrease in its protein expression in psoriatic skin biopsies compared to controls. Aki *et al.* [25] reported a reduced expression of TNFAIP3 mRNA in skin biopsies from psoriatic patients in both involved and uninvolved skin. Based on the finding that low TNFAIP3 expression is associated with increased susceptibility to inflammation, it can be concluded that decreased TNFAIP3 levels potentiates psoriasis susceptibility. Additionally, the fact that NF- κ B stimulates TNFAIP3 mRNA expression in response to inflammation confirms that low TNFAIP3 expression acts as a driver rather than a result of the inflammatory process seen in psoriasis [26]. One of the key factors causing inflammation during psoriasis is hyper-activated NF- κ B. NF- κ B is also known to be the primary regulator in psoriasis pathology, where multiple cell types, chemokines, and psoriasis-associated cytokines are dependent on the activation of NF- κ B signaling.

CONCLUSION

The present findings may provide additional evidence for the association of TNFAIP3 rs610604 (C / A), gene polymorphism with psoriasis susceptibility, and may serve as prognostic biomarkers.

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