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TNFα -308 and +489 polymorphisms in psoriatic arthritis patients at zagazig university hospitals.

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ABSRACT

Background: Psoriasis is a multifactorial disease with wide range of clinical manifestations. Musculoskeletal manifestations of psoriasis include a combination of axial disease, peripheral arthritis, enthesitis and dactylitis. The tumor necrosis factor α (TNFα) gene is proposed as a fundamental gene in psoriatic arthritis (PsA)**.** The aim of the work is to detect frequency of TNFα gene -308 and +489 polymorphisms genotypes among psoriatic and PsA patients and assess the risk for psoriasis and PsA. **Methods:** A case control study was conducted in Rheumatology and Rehabilitation Department, Zagazig University Hospitals on 96 subjects. They were divided into three equal groups (PsA, cutaneous only psoriasis (PsC) and control). Full history taking, clinical examination and assessment of PsA activity by composite psoriatic disease activity index (CPDAI) were done. Laboratory investigations included CBC, ESR, CRP, RF, kidney and liver function tests. Detection of TNFα gene -308 and +489 polymorphisms was performed using PCR RFLP (restriction fragment length polymorphism) technique. **Results:** Regarding TNF (+489) genotyping, AA and GA genotypes and A allele were more frequent in PsA and PsC patients than in controls. The A allele increased the risk for PsA and psoriasis by 8.4 and 5.9 folds respectively. TNF (+489) GA genotype was associated with higher activity of PsA. Regarding TNF (-308), there was no significant difference in genotypes frequency among three groups and no relation with PsA disease activity. **Conclusions:** TNF (+489) A allele carried risk for PsA and psoriasis and TNF (+489) GA genotype was associated with higher activity of PsA.

Key words: psoriasis, psoriatic arthritis, TNF polymorphism, PCR RFLP.

INTRODUCTION

Psoriatic arthritis has heterogenous clinical manifestations, PsA may affect peripheral joints, sacroiliac joints and the spine[1]. Psoriatic arthritis (PsA) occurs in approximately 30% of psoriasis patients. Understanding the etiology of PsA may facilitate intervention and allow risk prediction in the future[2]. TNFα single nucleotide polymorphisms (SNPs) are involved in psoriasis in various processes including: skin barrier functions suggesting that TNFα gene polymorphisms could be used to predict the risk of psoriasis and PsA [3].The aim of the work is to detect frequency of TNFα gene -308 and +489 polymorphisms genotypes among psoriatic and PsA patients and assess the risk for psoriasis and PsA**.**

METHODS

This case control study was conducted at Rheumatology and Rehabilitation Department and dermatology Department, Zagazig

University Hospitals. Informed consent was obtained from all participants and the study was approved by the research ethical committee of Faculty of Medicine Zagazig University. The work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans. This study included 96 subjects, they were divided into three equal groups. The first group had 32 PsA patients (diagnosed according to CASPAR criteria) [4] and the second group included 32 PsC patients. The third group included 32 apparently healthy volunteers. All patients were exposed to full history taking, full clinical examination. Psoriatic arthritis disease activity was assessed by using Composite Psoriatic Disease Activity Index (CPDAI). [5] Laboratory investigations included complete blood picture, ESR, CRP, RF, kidney and liver function tests.

Determination of *TNF-α -308 and TNF-α +489* gene polymorphisms

Blood DNA extraction was done using standard procedures. *TNF-α -308 and TNF-α +489* gene polymorphisms was conducted by polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP). The primers 5'-GGAGA-GAAGCAACTACAGAC-3'(forward)5'CACACTTAGTGAGCACCTTC 3' (reverse) for *+489* polymorphism. The amplified products (*G* allele at 111,159 and 281 bp fragment, and *A* allele at 159 and 392 bp fragments) were digested with *Tai I.* [6] Primers 5'- TTCCTGCATCCTGTCTGGAA -3' (forward) 5' AGCGGAAAACTTCCTTGGT-3' (reverse) for *-308* polymorphism. The amplified products (*G* allele at 137,103 and 88bp fragment, and *A* allele at 191 and 137bp fragments) were digested with *BsmfI.* [7]They were analysed by electrophoresis on a 4% agarose gel stained with 0.1% ethidium bromide. The digested fragments were visualized on a UV transilluminator.

Statistical analysis:

The collected information were coded, entered, presented, and analyzed by computer via a data base software program, Statistical Package for Social Science (SPSS) version 12.0.1 (SPSS, Inc., Chicago, IL, USA). Parametric variables were represented as the mean and standard deviation (SD), and nonparametric data expressed as median and range. Chi square (X^2) was used to detect relation between different qualitative variables. For quantitative variables one-way ANOVA (F) was used for normally distributed data, while Kruskal Wallis (KW) was used for comparison of median of more than two independent samples if they are not normally distributed. P value ≤ 0.05 means statistically significant. For risk assessment Odd`s ratio (OR) and confidence interval (95%CI) were also calculated.

RESULTS

Demographic and laboratory characteristics of PsA, PsC patients and controls: [**Table 1]** showed no significant difference among three groups as regards age, sex and family history. Females were 72% in PsA patients, 59% in PsC patients and 66% in control group. Positive family history was present in 12.5% of PsA patients and 21.9% of PsC patients. Laboratory investigations revealed that CRP and ESR among PsA group were higher than other two groups with statistically significant difference (p=0.0001 and p=0.0001) as shown in **[table 2]**.

Genotyping of the three studied groups: **[Table 3]** showed genotype and allele frequencies of TNF *(-308)* and *(+489)* polymorphisms in the three studied groups. The frequency of TNF (+489) AA genotype was significantly higher in PsA and PsC groups compared to control group (p=0.006 and p=0.04 respectively). The frequency of TNF (+489) GA genotype was higher significantly in PsA and PsC groups compared to control group (p=0.006 and p=0.02 respectively). Frequency of TNF (+489) A allele was higher with significant difference in PsA and PsC groups compared to control group (p<0.001 and p=0.001 respectively). The A (+489) allele increased the risk for PsA by 8.4 folds and for PsC by 5.9 folds. On the other hand, The frequency of TNF (-308) genotypes

and alleles had no significant difference among three studied groups.

Relation between TNF *(-308)* and *(+489)* genotypes and disease activity by CPDAI in PsA patients

[Table 4] showed that in PsA group, GA genotype of TNF(+489) polymorphism was associated with higher CPDAI than GG and AA genotypes with statistically significant difference (p=0.04).

Table 1. Demographic data and family history of the studied groups

	PsA group	PsC Group	Control group	Test	p
Age (years) Range Mean±SD	28-70 42.7±10.2	19-74 43.2±12.7	19-73 43.3±12.3	F	0.97
Sex no. (%) Females Males	 23(72) 9(28)	 19(59) 13(41)	 21(66) 11(34)	X^2	0.58
Family history: (1st and 2nd degree relatives) no.(%) -psoriasis: Psoriatic skin no.(%) Psoriatic arthritis no.(%) -RA no.(%)	 4(12.5) 3(9.3) 0 1(3.1)	 7(21.9) 6(18.75) 0 1(3.1)			

PsA: psoriatic arthritis, PsC: cutaneous only psoriasis, F: ANOVA test of significance, X^2: chi square test, no: number, SD: standard deviation, RA: rheumatoid arthritis.

Table 2. Laboratory investigations among three studied groups.

	PsA	PsC	Control	Test	P
WBCs (10^3/µL) mean± SD	8.1±2.5	8.2±2.2	8±2.1	F	0.94
Hb (g/dl) mean± SD	11.6±1.5	11.4±1.3	11.5±1.3	F	0.88
Platelet (10^3/ µL) mean± SD	262±58	246.5±52	247±53	F	0.4
ESR (mm/hr) mean± SD	41.7±13	28.9±9.1	18.5±6.4	F	0.0001
CRP (mg/dl) Median (range)	1(0.13-4.3)	0.4(0.2-1)	0.5(0.2-1.1)	KW	0.0001
RF titre (U/mL) Median (range)	9.5(4-30)	9(4-20)	9(4-7)	KW	0.905
ALT (U/L) Median (range)	20.5(5.6-67)	23.5(12-43)	23(10-44)	KW	0.441
AST (U/L) Median (range)	19.5(11-50)	22(10-38)	23.5(13-40)	KW	0.578
Albumin (g/dl) Median (range)	4(3.1-4.5)	4.2(3.4-4.7)	4.1(3.2-4.5)	KW	0.911
Total bilirubin(mg/dl) Median (range)	0.4(0.17-0.87)	0.53(0.14-1.1)	0.48(0.14-0.9)	KW	0.146

BUN (mg/dl) Median (range)	18(5-34)	20(10-40)	19(6-37)	KW	0.478
Creatinine(mg/dl) Median (range)	0.8(0.44-1.2)	0.9(0.4-1.5)	0.7(0.5-1.1)	KW	0.971

PsA: psoriatic arthritis, PsC: cutaneous only psoriasis, WBCs: white blood cells, Hb: hemoglobin, ESR: erythrocyte sedimentation rate, CRP: c-reactive protein, RF: rheumatoid factor, ALT: alanine transaminase, AST: aspartatetransaminase, BUN: blood urea nitrogen, dl: deciliter, mg: milligram, g: gram, µL: microliter, U: unit,L: liter, SD: standard deviation, no: number, F: ANOVA test, KW: Kruskal Wallis Test.

Table 3. Genotype and allele frequencies of TNF (-308) and (+489) SNP in the three studied groups.

	PsA no(%)	PsC no(%)	Control no(%)	P1	P2	P3
TNF (-308)						
Genotype						
GG	25(78.1)	23(71.9)	26(81.3)	1	1	1
GA	6(18.8)	7(21.9)	5(15.6)	1.0	0.70	0.95
AA	1(3.1)	2(6.3)	1(3.1)	1.0	0.95	0.97
Allele						
G	56(87.5)	53(83)	57(89)	1	1	1
A	8(12.5)	11(17)	7(11)	0.78	0.31	0.46
OR(95%CI)				0.86(0.3-2.5)	0.59(0.2-1.6)	0.69(0.3-1.5)
TNF (+489)						
Genotype						
GG	16(50)	19(59.3)	29(90.6)	1	1	1
GA	9(28.1)	8(25)	2(6.3)	**0.006**	**0.02**	0.62
AA	7(21.9)	5(15.6)	1(3.1)	**0.006**	**0.04**	0.45
Allele						
G	41(64.1)	46(71.9)	60(94)	1	1	1
A	23(35.9)	18(28.1)	4(6)	**<0.001**	**0.001**	0.34
OR(95%CI)				8.4(2.7-26.1)	5.9(1.9-18.6)	0.69(0.3-1.5)

SNP: single nucleotide polymorphism, PsA: psoriatic arthritis, PsC: cutaneous only psoriasis, P1:chi square test of significance between PsA and control, P2:chi square test of significance between PsC and control, P3:chi square test of significance between PsA and PsC, OR(95%CI): Odds ratio for A allele.

Table 4. Relation of genotypes of TNF(-308) and TNF(+489) and CPDAI in Psoriatic arthritis patients group.

Genotype		CPDAI	Test	P
		Mean±SD		
TNF(-308)	AA no=1	9	F	0.06
	GA no=6	6.3±2.3		
	GG no=25	8.5±1.8		
TNF(+489)	AA no=7	6.2±1.3	F	0.04
	GA no=9	9±1		
	GG no=16	8.2±2.3		

F :ANOVA test, CPDAI: composite psoriatic disease activity index, SD: standard deviation

DISCUSSION

This study included 96 subjects, 32 of them were suffering from PsA. Their ages ranged from 19 to 74 years and females represented 72%. In the study [8] PsA patients aged from 18 to 62 years and females were 46.7%. Second group of subjects were 32 with only cutaneous manifestations psoriatic patients (PsC) with their ages ranging from 19 to 74 years and females were 59%. Similarly, [9]conducted a study on PsC patients with age ranging from 19 to 70 years and females represented 62% of their patients, the other 32 subjects (control group) were apparently healthy volunteers.

Positive family history for psoriasis or PsA was present in 12.5% in PsA group and 21.9% in PsC group. This is consistent with[10]who detected family history of psoriasis in 20.7% of psoriatic patients,while in the study of [11] approximately 40% of patients had a family history of psoriasis or PsA.

ESR and CRP were higher significantly among PsA group than PsC and control groups. This was in agreement with[12] who stated that inflammatory markers are associated with arthritis in psoriatic patients.

The current study showed that the frequency of TNF (+489) AA genotype was significantly higher in PsA and PsC patients than in controls. Also, the A allele frequency was higher in PsA and PsC groups than in control group (35.9%, 28.1% and 6% respectively). The A(+489) allele increased the risk for PsA by 8.4 folds and increased the risk for PsC by 5.9 folds. Similarly [13] reported that A allele of TNF (+489) was significantly associated with PsA susceptibility as frequency of A allele in controls 13.9% compared to 24.6% in PsA with significant difference.

Current study showed that frequency of TNF (-308) A minor allele was 11% in control group and 12.5% in PsA group and had no significant difference between three studied groups regarding genotype and allele frequencies of TNF (-308) SNP. In addition, the previous findings were consistent with [14] as they observed no significant difference in genotype frequencies of TNF (-308) between the control and the PsA patient populations. Also, a canadian research on TNF (-308) polymorphism was conducted in two populations (Newfoundland and Toronto) and it found that A allele frequency was 18.4% in Newfoundland PsA and 18.9% in control. A

allele frequency was 16% in Toronto PsA and 19.3% in control with no significant difference [15].These findings were quite different from the results of [16]who found that the TNF -308 A allele was overrepresented in psoriatic patients than controls.

Concerning disease activity assessed by CPDAI among PsA patients, GA genotype of TNF(+489) polymorphism was associated with higher activity by CPDAI than GG and AA genotypes with statistically significant difference (p=0.04). This results was in agreement with [17].

CONCLUSIONS

The TNF (+489) A allele increased the risk for PsA and psoriasis by 8.4 and 5.9 folds respectively. On the other hand, TNF (-308) genotypes and alleles had no significant difference between three groups. The TNF (+489) GA genotype was associated with higher PsA disease activity by CPDAI. Further larger studies are needed to assess association of TNF polymorphisms with disease susceptibility, activity and severity and its impact on response to treatment with anti TNF therapy.

Conflict of Interest: Nothing to declare

Financial Disclosure: Nothing to declare

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