



Tumor necrosis factor- α gene promoter –308 and –238 polymorphisms and its serum level in psoriasis

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ABSTRACT

Background: Psoriasis is a chronic, immune-mediated, inflammatory skin disease affecting genetically predisposed individuals and requiring long-term treatment. The etiology of psoriasis is not fully understood. This article aimed to determine association between genetic polymorphisms in tumor necrosis factor- α (TNF- α) promoter –308 (rs1800629) and –238 (rs 361,525) and its serum level in psoriasis patients.

Methods: The study was conducted on 70 patients with psoriasis and 70 age and sex-matched, healthy individuals. All patients were subjected to history taking and complete medical examination. The polymorphisms of TNF- α promoter gene –308 (rs1800629) and –238 (rs 361,525) were detected by real time PCR and Serum levels of TNF- α were measured by ELISA technique.

Results: AG polymorphism and A allele of TNF- α –238 G/A (rs 361,525) were significantly more in patients than controls, whereas AG polymorphism and A allele of TNF- α –308 G/A (rs1800629) were significantly more in controls than patients. There were significant high levels of TNF- α in serum of patients in comparison to controls.

Conclusions: The AG polymorphism and A allele of TNF- α –238G/A (rs 361,525) may act as a risk factor for occurrence of psoriasis, whereas AG polymorphism and A allele of TNF- α –308G/A (rs1800629) may have protective role. There is pivotal role of TNF- α as a pro-inflammatory mediator in pathogenesis of psoriasis.

1. Introduction

Psoriasis (PS) is a chronic inflammatory dermatological condition, associated with both physical and psychological burdens [1]. Nowadays it has been recognized as a systemic inflammatory disorder, characterized by remission and relapse [2]. PS vulgaris, the commonest form of PS, accounts for 70–80% of psoriatic patients. The patients are presented with sharply demarcated round, oval, or nummular (coin-sized) plaques with loosely adherent silvery white scales, specially affecting extensor surfaces rather than flexors. The lesions usually begin as erythematous papules, extend peripherally, and coalesce to form plaques [3]. Psoriasis is a common disorder worldwide. The prevalence of psoriasis in Egypt, an African country with a Caucasian population, ranges 0.19–3% [4]. (see Tables 1–5)

Pathogenesis of psoriasis still controversial, but it can be explained by coexistence and interaction between three elements: genetic susceptibility, immunological (innate/humoral) imbalance and environmental triggering factors [5].

Tumor Necrosis Factor- α (TNF- α), one of pro-inflammatory cytokines, is a 17-kDa cytokine, which is produced by various cell types, including activated macrophages, lymphocytes, fibroblasts, and keratinocytes, in response to various infectious and non-infectious stimuli [6]. It has wide range of functions as it plays a physiological protective apoptotic role against some tumours (hence the name) and some types of infections when expressed within normal level, but sometimes it has a harmful destroying effect when highly expressed as in inflammatory, autoimmune and cell proliferative disorders [7].

Related studies showed that there are different single nucleotide polymorphisms (SNPs) in different genes of cytokines that can cause changes in cytokines transcriptional levels and/or their activities, which in turn affect path and pathogenesis of different diseases [8]. The TNF- α gene is found on human chromosome 6 (6p21.3) within the region encoding the Major Histocompatibility Complex (MCH), HLA in human, It is a region of great polymorphic variation with up to 44 polymorphisms reported [9]. TNF- α polymorphisms have been investigated in different diseases and especially whenever there is an HLA association

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Table 1Comparison between the two studied groups according to TNF- α gene-238 (rs361525) polymorphisms.

	Patients(n = 70)		Control(n = 70)		Un adjusted[OR (95% CI)]	p	Adjusted [OR (95% CI)]	p
	No.	%	No.	%				
Gene-238								
GG	35	50.0	48	68.6	1.000		1.000	
AG	24	34.3	19	27.1	1.732 (0.82–3.64)	0.147	2.334 (1.03–5.27)	0.041*
AA	11	15.7	3	4.3	5.029 (1.31–19.38)	0.019*	8.958 (1.95–41.08)	0.005*
HWE	0.062		0.531					
Dominant								
GG	35	50.0	48	68.6	1.000		1.000	
AG + AA	35	50.0	22	31.4	2.182 (1.1–4.34)	0.026*	3.039 (1.41–6.55)	0.005*
Recessive								
GG + AG	59	84.3	67	95.7	1.000		1.000	
AA	11	15.7	3	4.3	4.164 (1.11–15.64)	0.035*	6.269 (1.46–26.84)	0.013*
Over dominant								
GG + AA	46	65.7	51	72.9	1.000		1.000	
AG	24	34.3	19	27.1	1.400 (0.68–2.88)	0.360	1.690 (0.78–3.64)	0.181
Allele								
G	94	67.1	115	82.1	1.000		1.000	
A	46	32.9	25	17.9	2.251 (1.288–3.933)	0.004*	3.031 (1.634–5.621)	<0.001*

OR: Odds ratio CI: Confidence interval.

*: Statistically significant at $p \leq 0.05$.

Adjusted OR by age and sex.

Table 2Comparison between the two studied groups according to TNF- α Gene-308 (rs1800629) polymorphisms.

	Patients(n = 70)		Control(n = 70)		Un adjusted [OR (95% CI)]	p	Adjusted [OR (95% CI)]	p
	No.	%	No.	%				
Gene-308								
GG	54	77.1	33	47.1	1.000		1.000	
AG	13	18.6	25	35.7	0.318 (.14–0.71)	0.005*	0.184 (.07–0.45)	0.001*
AA	3	4.3	12	17.1	0.153 (0.04–0.58)	0.006*	0.080 (0.02–0.33)	0.001*
HWE	0.081		0.072					
Dominant								
GG	54	77.1	33	47.1	1.000		1.000	
AG + AA	16	22.9	37	52.9	0.264 (0.13–0.55)	0.001*	0.148 (.06–0.34)	0.001*
Recessive								
GG + AG	67	95.7	58	82.9	1.000		1.000	
AA	3	4.3	12	17.1	0.216 (0.06–0.81)	0.022*	0.156 (.04–0.59)	0.006*
Over dominant								
GG + AA	57	81.4	45	64.3	1.000		1.000	
AG	13	18.6	25	35.7	0.411 (0.19–0.89)	0.025*	0.303 (.13–0.69)	0.005*
Allele								
G	121	86.4	91	65.0	1.000		1.000	
A	19	13.6	49	35.0	0.292 (.16–0.53)	0.001*	0.198 (.11–0.37)	0.001*

OR: Odds ratio CI: Confidence interval.

*: Statistically significant at $p \leq 0.05$.

Adjusted OR by age and sex.

with the disease e.g. insulin dependent diabetes mellitus (IDDM) and rheumatoid arthritis (RA) [10,11].

Two single-nucleotide polymorphisms involving G/A transitions in the promoter region of TNF- α gene at the –238 (rs 361,525) and –308 (rs1800629) sites have been shown to influence TNF- α expression and have been associated with the occurrence of many diseases [12,13].

In this study we aimed to determine association between genetic polymorphisms in tumor necrosis factor- α (TNF- α) promoter –308 (rs1800629) and –238 (rs 361,525) and its serum level in psoriasis patients.

2. Materials and methods

This study was completed by coordination between Dermatology,

Andrology & Sexually Transmitted Diseases (STDs) and Medical Biochemistry and Molecular Biology Departments, Faculty of Medicine, Menoufia University. This case control study enrolled 70 patients with psoriasis vulgaris (group 1) and 70 age and gender matched healthy volunteers served as control group (group 2). Cases were selected from the Dermatology And Leprosy Kaphr Elsheikh Hospital during the period from September 2019 to December 2019. Study was conducted in accordance with the Declaration of Helsinki. A written consent form approved by Local Institutional Ethical Committee of Menoufia Faculty of Medicine was taken from every participant before the study initiation. Patients were either newly diagnosed with no history of treatment or stopped systemic and topical treatment except emollients for at least 3 months before samples taking. Patients with any dermatological diseases other than psoriasis, systemic lupus, rheumatoid, malignancy and

Table 3
Relation between TNF- α gene –238 (rs361525) polymorphisms and clinical data in patients group.

	Gene-238						Test of Sig.	p
	GG (n = 35)		AG (n = 24)		AA (n = 11)			
	No.	%	No.	%	No.	%		
Sex								
Male	32	91.4	21	87.5	9	81.8	$\chi^2=1.146$	$^{MC}p=0.613$
Female	3	8.6	3	12.5	2	18.2		
Age (years)								
Min. – Max.	11.0–80.0		19.0–78.0		22.0–71.0		H = 0.248	0.883
Mean \pm SD.	44.40 \pm 15.82		45.21 \pm 17.52		47.18 \pm 15.69			
Median	49.0		44.50		50.0			
BMI (kg/m²)								
Min. – Max.	18.90–33.0		20.20–33.70		24.40–32.0		H=1.108	0.575
Mean \pm SD.	26.01 \pm 3.65		26.27 \pm 3.40		27.30 \pm 2.20			
Median	25.90		26.90		27.0			
HTN								
No	26	74.3	16	66.7	8	72.7	$\chi^2=0.416$	$^{MC}p=0.812$
Yes	9	25.7	8	33.3	3	27.3		
DM								
No	32	91.4	22	91.7	11	100.0	$\chi^2=0.672$	$^{MC}p=1.000$
Yes	3	8.6	2	8.3	0	0.0		
Onset								
Early	23	65.7	15	62.5	5	45.5	$\chi^2=1.468$	0.480
Late	12	34.3	9	37.5	6	54.5		
Family history								
Negative	31	88.6	20	83.3	9	81.8	$\chi^2=0.766$	$^{MC}p=0.727$
Positive	4	11.4	4	16.7	2	18.2		
Duration (years)								
Min. – Max.	0.08–21.0		0.08–30.0		1.0–35.0		H = 0.391	0.822
Mean \pm SD.	7.58 \pm 6.22		8.34 \pm 8.27		9.55 \pm 9.47			
Median	5.0		6.50		7.0			
PASI score								
Min. – Max.	0.60–41.10		1.60–43.90		6.70–38.60		H=27.581*	0.001*
Mean \pm SD.	6.95 \pm 8.80		18.31 \pm 10.63		17.95 \pm 9.23			
Median	4.0		14.65		13.90			
SerumTNF- α (pg/ml)								
Min. – Max.	129.0–256.10		148.90–275.80		166.90–259.0		F=10.638*	0.001*
Mean \pm SD.	175.38 \pm 28.0		205.88 \pm 39.42		219.38 \pm 29.08			
Median	174.0		212.50		218.70			

χ^2 : Chi square test MC: Monte Carlo H: H for Kruskal Wallis test.

*: Statistically significant at $p \leq 0.05$.

Blood transfusion in the last 6 months were excluded from this study. Each of the selected cases was subjected to the following: complete history taking including history of disease (age of onset, duration, site, presence of itching and koebnerization) and family history of psoriasis, hypertension (HTN), DM, smoking, and alcohol consumption, complete general examination and dermatological examination (clinical assessment of the disease severity by a widely used tool for the measurement of severity of psoriasis. PASI score was calculated as described in the study by Langley and Ellis, a higher PASI score presents a greater degree of psoriatic severity [14,15]. All subjects underwent these laboratory investigations: estimation of serum level of TNF- α by using a double antibody sandwich ELISA kit provided by Krishgen Biosystem (Ray-Biotech, USA), followed the manufacturer's instructions by using standard curve and detection of TNF- α promoter –308 (rs1800629) and –238 (rs 361,525) gene polymorphisms were detected by real time PCR.

2.1. Methods

5 ml of venous blood were taken by trained laboratory technician, out of which 2 ml were taken in a sterile EDTA coated vials then stored at –20 for genomic DNA extraction and the remaining 3 ml were centrifuged at 4000 rpm for 5min then serum was taken out and stored at –80 °C till analysis for estimation of TNF- α level.

2.2. Genotyping of TNF- α promoter –308 (rs1800629) and –238(rs 361,525) gene polymorphisms

DNA Extraction from whole blood was performed by Gene JET Genomic DNA Purification Kit (Thermo Scientific, Lithuania) and quantified by a spectrophotometer. Isolated DNA was stored at –20 °C for future use.

TaqMan genotyping and allelic discrimination assay kits were applied for sample analysis utilizing the 7500 Real-time PCR system (Applied Biosystems, Foster City, CA, USA). The provided fluorescent labeled TaqMan probes were sequenced as:

*GGCCCAGAAGACCCCTCGGAATC [A/G] GAGCAGGGAG-GATGGGGAGTGTGAG for TNF- α -238 (G/A) (rs 361,525) (VIC for allele A and FAM for allele G).

*GAGGCAATAGGTTTTGAGGGGCATG [A/G] GGACGGGGTT-CAGCCTCCAGGTCC for TNF- α -308 (rs1800629) (VIC for allele A and FAM for allele G).

To detect the selected SNPs, real-time PCR was set with a reaction volume of 20 μ l using 10 μ l of TaqMan Genotyping Master Mix, 1.25 μ l of 20X SNP, 3.75 μ l of nuclease-free water, and 5 μ l of the extracted DNA. The reaction was completed in 96-well plates and proceeded as described: 50 °C for 1 min (pre-PCR read), followed by 10 min at 95 °C then 45 cycles of 15 s for denaturation at 94 °C, 60 s for primer annealing

Table 4
Relation between TNF- α gene –308 (rs1800629) polymorphisms and clinical data in patients group.

	Gene-308						Test of Sig.	p
	GG (n = 54)		AG (n = 13)		AA (n = 3)			
	No.	%	No.	%	No.	%		
Sex								
Male	47	87.0	12	92.3	3	100.0	$\chi^2=0.323$	$MC_p=1.000$
Female	7	13.0	1	7.7	0	0.0		
Age (years)								
Min. – Max.	11–80		21–55		22.0–70.0		H = 1.668	0.434
Mean \pm SD.	45.72 \pm 16.57		41.54 \pm 13.11		49.67 \pm 24.83			
Median	47.0		49.0		57.0			
BMI (kg/m²)								
Min. – Max.	18.90–33.70		19.80–28.10		22.60–24.90		H=11.227*	0.004*
Mean \pm SD.	26.94 \pm 3.43		24.21 \pm 2.27		24.03 \pm 1.25			
Median	27.35		25.0		24.60			
HTN								
No	37	68.5	10	76.9	3	100.0	$\chi^2=1.069$	$MC_p=0.679$
Yes	17	31.5	3	23.1	0	0.0		
DM								
No	50	92.6	12	92.3	3	100.0	$\chi^2=0.506$	$MC_p=1.000$
Yes	4	7.4	1	7.7	0	0.0		
Onset								
Early	33	61.1	8	61.5	2	66.7	$\chi^2=0.209$	$MC_p=1.000$
Late	21	38.9	5	38.5	1	33.3		
Family history								
Negative	46	85.2	11	84.6	3	100.0	$\chi^2=0.294$	$MC_p=1.000$
Positive	8	14.8	2	15.4	0	0.0		
Duration (years)								
Min. – Max.	0.08–35.0		0.08–15.0		0.08–20.0		H = 1.892	0.388
Mean \pm SD.	8.60 \pm 7.71		5.86 \pm 5.60		10.03 \pm 9.96			
Median	7.0		3.0		10.0			
PASI score								
Min. – Max.	0.60–43.90		1.20–41.10		1.40–11.60		H = 5.147	0.076
Mean \pm SD.	13.81 \pm 10.95		9.05 \pm 11.15		5.47 \pm 5.40			
Median	11.65		4.0		3.40			
SerumTNF- α (pg/ml)								
Min. – Max.	130.20–275.80		129.0–211.0		144.0–181.0		F = 5.894*	0.004*
Mean \pm SD.	200.44 \pm 35.56		167.23 \pm 25.51		165.0 \pm 19.0			
Median	199.25		165.0		170.0			

χ^2 : Chi square test MC: Monte Carlo H: H for Kruskal Wallis test.

*: Statistically significant at $p \leq 0.05$.

Table 5
Comparison between the two studied groups according to serum level of TNF- α

TNF- α in serum (pg/ml)	Patients(n = 70)	Control(n = 70)	t	p
Min. – Max.	129.0–275.80	16.50–65.90	28.142*	0.001*
Mean \pm SD.	192.75 \pm 36.78	37.56 \pm 14.89		
Median (IQR)	193.10 (165.0–215.0)	38.0 (25.40–49.50)		

U: Mann Whitney test.

*: Statistically significant at $p \leq 0.05$.

at 50 °C and 2 min for extension at 72 °C and 1 min at 60 °C for post-PCR.

2.3. Statistical analysis

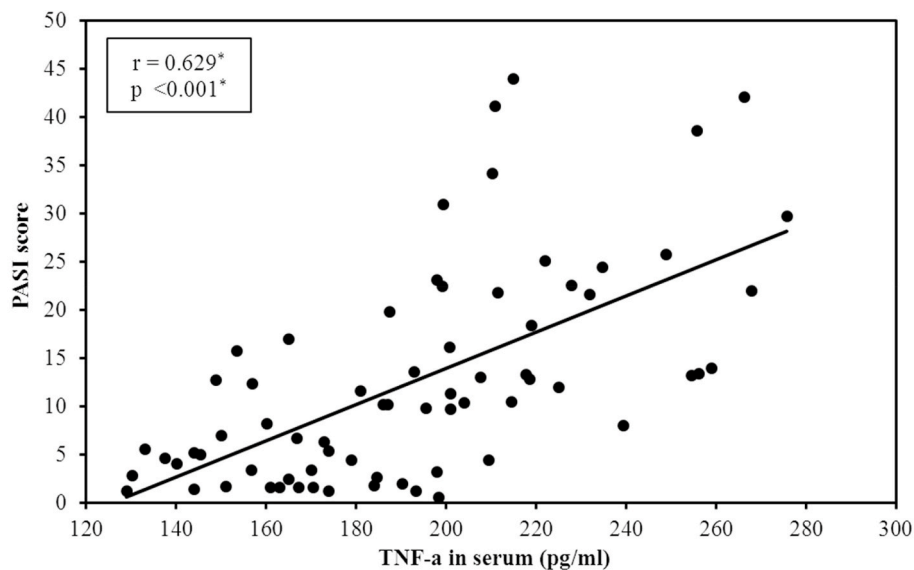
Data were analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). The student's t-test was used to compare between two groups regarding the quantitative variables, the chi-square (χ^2) test for qualitative data, and the Mann-Whitney test for the nonparametric variables. For comparisons between more than two groups, ANOVA and the Kruskal-Wallis test were used for normally and not normally distributed variables, respectively. Odd ratio (OR) with

95% Confidence Interval was used to calculate the ratio of the odds of an event occurring in one risk group to the odds of it occurring in the non-risk group. The population of the studied sample was explored to find its equilibrium with Hardy-Weinberg equation. Spearman coefficient to correlate between two not normally distributed quantitative variable. The significance of the results was referred to $P \leq 0.05$.

3. Results

Table (1) shows genotype frequencies and allelic distribution of TNF- α –238 (rs361525) gene in patients and controls. The allele A frequency was found to be significantly higher among patients [32.85% (46/140)] as compared with controls [17.9% (25/140)], adjusted OR (95%CI) = 3.031 (1.634–5.621), ($P = 0.001$). It was observed that AA and AG genotypes were higher in patients [15.7% (11/70) and 34.3% (24/70)] than in control group [4.3% (3/70) and 27.1% (19/70)] respectively, with OR (95%CI) = 2.334 (1.03–5.27), $P = 0.005$, and OR (95%CI) = 8.958 (1.95–41.08), $P = 0.041$.

Table (2) shows the allele and genotype frequencies of TNF- α –308 (rs1800629) gene in patients and controls. The allele A frequency was found to be significantly higher in controls [35% (49/140)] as compared with cases [13.57% (19/140)]. OR (95%CI) = 0.198 (0.11–0.37), ($P = 0.001$). It was observed that AA and AG genotypes were significantly



r: Pearson coefficient

*: Statistically significant at $p \leq 0.0$

Fig. 1. Correlation between TNF- α level in serum and PASI score in patients group.

higher in controls [17.1% (12/70) and 35.7% (25/70)] than in cases [4.3% (3/70) and 18.6% (13/70)] respectively. OR (95%CI) = 0.080 (0.02–0.33), ($P = 0.001$) and OR (95%CI) = 0.184 (0.07–0.45), ($P = 0.001$) respectively.

Table (3) shows the relation between TNF- α gene –238 (rs361525) polymorphisms and clinical data in patients group. TNF- α -238 (rs361525) gene polymorphisms showed no significant differences between cases and controls concerning gender, age, BMI, hypertensive, DM, onset of disease, disease and duration, while AA and AG genotype showed significantly higher PASI score than GG genotype ($P = 0.001$). TNF- α serum level was higher with AG and AA genotypes than with GG ($P = 0.001$).

Table (4) shows the relation between TNF- α gene –308 (rs1800629) polymorphisms and clinical data in patients group. BMI was significantly increased in GG genotype than AG and AA genotypes ($P = 0.004$). TNF- α serum level was higher with GG genotype than AG and AA ($P = 0.004$).

Table (5) shows comparison between the two studied groups according to serum level of TNF- α . TNF- α serum level in psoriasis patients was significantly elevated (192.75 ± 36.78 pg/ml) as compared with controls (37.56 ± 14.89) ($P = 0.001$).

In addition, significant positive correlation between TNF- α serum level and disease severity (PASI score) was observed ($P = 0.001$) Figure (1).

4. Discussion

Psoriasis is a chronic and recurrent inflammatory dermatological insult that is characterized by hyper proliferative keratinocytes and infiltrating immune cells, including T cells, dendritic cells, macrophages and neutrophils [16].

Our study reported that TNF- α serum level is higher between patients group than control one and this is inconsistency with Wang L et al., 2019 and Yost J et al., 2009 who stated that TNF- α , a proinflammatory cytokine, plays an important role in pathogenesis of psoriasis, as it stimulates the nuclear factor (NF)- κ B signal pathway [17], which influences cell survival, proliferation and anti-apoptotic impact of

lymphocytes and keratinocytes. In expansion, TNF- α fortifies keratinocytes to deliver IL-8, which results in micro abscess formation by increasing neutrophil recruitment process in psoriasis. TNF- α motivates Th17 to create pro-inflammatory cytokines through the NF- κ B pathway in psoriasis, and blockade of the NF- κ B pathway leading to loss of IL-17 A synthesis from CD4⁺ T cells. TNF- α production is increased in psoriatic lesions, because of this TNF suppressors are considered to be the latest revolution in treatment of psoriasis [18].

Its responsible gene is present in the short arm of chromosome 6 in the major histocompatibility complex class III region. Most polymorphisms of TNF- α gene are located in its promoter region and they are thought to affect the susceptibility and/or severity of different human diseases [19].

This review summarized the association between genetic polymorphisms in tumor necrosis factor- α (TNF- α) promoter gene –238 (rs 361,525) and –308 (rs1800629) in psoriasis patients.

The current study showed significant differences between psoriatic cases and healthy controls as regarding to TNF- α gene promoter –238 (rs 361,525) and –308 (rs1800629) polymorphisms. In agreement with our study Rahman et al., 2006 [20] and Zhuang et al. 2011 [21] revealed that TNF- α gene promoter –238 (rs 361,525) polymorphism is accompanied by increased risk of psoriasis, this is can be explained by increase in mutant A allele and AG, AA in psoriatic patients as compared with the control group, which confirms that TNF- α –238 (rs 361,525) AG polymorphism is associated with an increased risk for psoriasis.

AA and AG genotypes showed higher PASI score and higher TNF- α serum level than GG genotype which refers to great association between disease severity and –238 (rs 361,525) gene polymorphism as concluded in some studies [22]. Our study states that TNF- α gene promoter –238 (rs 361,525) polymorphism is accompanied by elevated PASI score and serum TNF- α level and not associated with BMI, age, gender nor duration of disease. This is conflicted with some studies that observed these differences regarding gender [23] and Reich K et al. [24] who also revealed an association between –238 (rs 361,525) gene polymorphism and early onset psoriasis.

As regards TNF- α –308 (rs1800629) gene, our results revealed increase in A allele, and AA and AG genotypes among control group if

compared with patients group, while G allele and GG genotypes are more in patients group than control one, which indicates that GG genotype is risky, while AA and AG are protective ones. This is explained, as the risky genotype is associated with higher TNF- α level, higher PASI score and BMI than AA and AG genotypic ones. Confirming the role of weight loss in lowering proinflammatory cytokines serum level that improves symptoms of the disease.

In contrast with our results, some earlier studies had found a higher frequency of TNF- α -308 GG in moderate to severe psoriasis patients [25]. In agreement with our results, some earlier studies had found a higher frequency of TNF- α -308 G allele in patients with early-onset psoriasis [24,26]. However, other investigators reported no difference in the distribution of TNF- α alleles or genotypes between patients and controls [27].

The difference in severity level (PASI) was found to be significantly correlated with serum TNF- α level ($P = 0.001$), proving the role of these cytokines as main parameters for disease severity. The study by Takahashi et al., 2010 [28] revealed that serum level of TNF- α was markedly elevated in psoriatic patients as compared with healthy controls; elevated levels were also correlated with PASI.

Our recommendations are:

- 1 - Further studies involving different ages and different clinical varieties of the disease are needed to expand and validate current findings
- 2 -Future studies on different populations and ethnicities.
- 3 -The clinical usefulness of TNF- α blockers in psoriasis treatment needs more expanded studies.

Ethical approval

This study was conducted in accordance with the Declaration of Helsinki. A written consent form approved by Local Institutional Ethical Committee of Menoufia Faculty of Medicine was taken from every participant before the study initiation. The IRB number is: 191119DERM37.

Author statement

Magda M Hagag selected the study design.

Nesreen G. Elhelbawy, (the corresponding author), performed the laboratory investigations and the molecular analysis.

Mai M Ghazy was a major contributor in writing the manuscript.

All authors participate in writing and revision of the paper and approved the final manuscript.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

All authors declare no conflict of interest.

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