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In severe acne vulgaris, TNF-α gene variants are connected to increased TNF-α gene expression and insulin resistance

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Abstract

Background: Acne vulgaris (AV) is a chronic inflammatory skin condition affecting the pilosebaceous unit, commonly presenting as comedones, papules, pustules, or nodules on the face, upper limbs, torso, and back, with comedones formation being the primary pathology leading to disfiguring inflammation, hyperpigmentation, scarring, and psychological impact.

Aim: The purpose of this study was to investigate the significance of two genetic variants in the promoter region of the tumor necrosis factor-alpha (TNF-α) gene and their association with insulin resistance (IR) in acne patients. To understand how these variants contribute to AV and its associated IR.

Subjects and methods: An analytical cross-sectional study with a case-control design and research evaluation was carried out on 87 AV patients and 73 healthy volunteers. The medical histories of both groups were obtained, as well as the severity and duration of inflammation among acne sufferers, as well as demographic data. Biochemical analysis was performed on both sets of participants, including fasting blood glucose levels, insulin levels while fasting, IR, and serum TNF-α. PCR-RFLP analysis identified −863 G > A (rs1800630) and −308 G > A (rs1800629) variations, and

Abbreviations:: AA, Alopecia areata BMI: body mass index; AV, Acne vulgaris; CRP, C-reactive protein cDNA: complementary DNA; DNA, Deoxyribonucleic acid; ELISA, enzyme-linked immunosorbent test; PCR, polymerase chain reaction; VDR, Vitamin D receptor.

real-time PCR analysis evaluated TNF-α gene expression in both patients and healthy people.

Results: Acne patients exhibited significantly higher levels of IR, fasting glucose, fasting insulin, serum TNF-α, and TNF-α folding change, when compared to healthy controls. The co-dominant model for −863 G > A and −308 G > A variants exhibited significant variations between the two groups. Severe acne patients who had the A/A genotype for −308 variants exhibited higher levels of IR, serum TNF-α, and TNF-α folding change. Highly significant positive linear correlation between IR, serum TNF-α, and TNF-α folding change in severe AV.

Conclusion: There is a correlation between AV, especially severe acne, and the −863 G > A and −308 G > A polymorphism, which influences TNF-α gene expression and serum TNF-α levels.

KEYWORDS
acne vulgaris, insulin resistance, serum TNF-α, TNF-α gene, TNF-α gene expression

1 | INTRODUCTION

Multiple factors contribute to the development of acne vulgaris (AV), including excessive sebaceous production, inflammation, and hormonal factors.[1]

In acne, insulin has a key role in inducing androgen production, which in turn leads to hyperkeratinization, excessive sebum production, and sebaceous gland proliferation. So, the etiology and severity of acne may be influenced by insulin.[2]

There is increasing interest in the potential connection between AV and insulin resistance (IR). Limited information is currently available, but it is known that patients with AV often have elevated insulin serum levels. Both acne and IR involve similar hormonal and signal transduction pathways.[3]

Interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF-α) are markers of inflammation that serve an important role in the growth and progression of AV. They influence hyperkeratinization, the first observable stage of microcomedone growth. Injury is caused by aberrant generation of lipids and IL-1 release from epithelial cells, which starts the acne formation cascade. IL-1 influences keratinocytes and stimulates the generation of TNF-α, which activates them. The function of primary and secondary inflammatory cytokines in acne etiology is still debated, as research on genetic associations has demonstrated. The TNF-α superfamily and its receptors have a variety of implications for skin problems. Genetic variables, specifically SNPs in the TNF-α gene promoter, may influence TNF expression and the occurrence of acne lesions.[4]

The goal of this research is to evaluate the significance of the −863 G > A (rs1800630) and −308 G > A (rs1800629) variants in the modulation of TNF-α gene expression, as well as to explore the potential association between the TNF-α gene and IR in acne patients. To learn more about how these genetic variations work and may play a role in the etiology of AV and its associated IR by examining the relationship between the TNF-α gene and IR.

2 | SUBJECTS AND METHODS

2.1 | Subjects

This study is a case-control study that involved 87 acne patients and 73 healthy controls. The study was conducted for a year in a dermatology outpatient department and followed guidelines set by the Helsinki Declaration. The research was granted permission by Shaqra University's Ethics Committee; the protocol reference number is ERC_SU_20220088, and individuals provided written consent. The research incorporated a complete medical history for both acne patients and controls, including demographic information, acne history, and medication usage.

The severity of acne was graded as mild, moderate, or severe. Mild acne is defined as (at least 20 comedones, 15 inflammatory lesions, or a total lesion count of 30). Moderate acne is distinguished by the presence of 20–100 comedones, 15–50 inflammatory lesions, or a total lesion count of 30–125. Severe acne (more than five pseudocysts, a total comedones count of more than 100, a total inflammatory count of more than 50, or a total lesion count of more than 125).[5]

Acne individuals between 15 and 45 of any gender who have not undergone acne therapy in the previous six weeks are eligible.

Being pregnant, nursing, usage of oral contraceptives, or being postmenopausal are all exclusion criteria. Individuals with other systemic diseases, those on regular treatment for various illnesses, and those who consumed isotretinoin by mouth in the last 3 months have been removed from the trial.

3 | DNA EXTRACTION AND GENOTYPE ANALYSIS

3.1 | DNA extraction and purification

Blood specimens were obtained using Na2EDTA as a coagulation inhibitor. According to the manufacturer's guidelines for blood methodology, hereditary deoxyribonucleic acid was isolated from 200 μL of entire blood utilizing the QIAamp® DNA BloodMini Kit (Qiagen, Hilden, Germany).

3.2 | Genotypes analysis of −863 G > A variants (rs1800630)

−863G > A genotype of the TNF-α gene detected by PCR−RFLP as previously described by *Veloso* et al. in their research.[6] The forward primer F:5-GGCTCTGAGGAATGGGTTAC-3′and reverse primer R:5′-CTACATGGCCCTGTCTTCGTTACG-3′ The primer sequence was subjected to a blasting procedure at the URL https://blast.ncbi.nlm.nih.gov/Blast.cgi. The PCR product was 126 bp digested by BsaAI restriction enzyme (NEB) at 37°C for 8 h, electrophoresed on 3% agarose gel stained with ethidium bromide. The 126 bp band corresponded to the C wild-type C allele and a set of 103 bp and 23 bp bands corresponded to the variant A allele.

3.3 | Genotypes analysis of −308 G > A (rs1800629)

−308G > A genotypes of TNF-α gene detected by PCR−RFLP as previously described by Banday et al described[7] the forward primer sequence F:5′ -GGAGGCAATAGGTTTTGAGGGCCAT-3′ and the reverse primer sequence R:5′-CTGTCT-CGGTTTCTTCTCCATGGCG-3′.The primer sequence was subjected to a blasting procedure at the URL https://blast.ncbi.nlm.nih.gov/Blast.cgi. The amplified PCR product 195 bp was digested using NcoI restriction enzyme (NEB). The digested PCR products were checked on 3% agarose gel. A/A genotype undigested (195 bp) while G/G digested into (173 and 22 bp) the G/A yielded three fragments (195 bp, 173 bp, and 22 bp).

4 | RNA EXTRACTION GENE EXPRESSION

4.1 | RNA extraction and purification

Total RNA was extracted from blood samples using a Qiagen QIAamp RNA Blood Mini Kit (Catalog number: 52304), and RNA purity was checked using a Nanodropper 2000. (ThermoScientific).

4.2 | cDNA synthesis

The process of reversing RNA into complementary DNA (cDNA) is achieved through the utilization of the cDNA Reverse Transcription Kit, which is manufactured by Invitrogen, a prominent company in the field of Life Sciences.

4.3 | Quantitative real-time PCR

Quantitative real-time PCR was performed using a Bio-Rad Cycler, Maxima SYBER Green Q PCR Master Mix, cDNA, and specific primers TNF-α gene F: 5′ CCCAGGCAGTCAGATCATCTTC-3′, R: 5′-AGCTGCCCCTCAGCTTGA-3′. The primer sequence was subjected to a blasting procedure at the URL https://blast.ncbi.nlm.nih.gov/Blast.cgi.

The PCR involved 40 cycles of denaturation, annealing, and extension. Normalization was done based on the expression level of the internal control gene beta-actin and specific primer sequences were used for beta-actin and TNF-α gene.[8]

The relative mRNA expression of the candidate gene is calculated by averaging the threshold cycle (Ct) numbers obtained from triplicate amplification reactions, and the magnitude of change in mRNA expression is determined using the standard $2^{-(\Delta\Delta\,ct)}$ method.[9]

4.4 | Biochemical analysis

Serum glucose determination was conducted using a kit from Bio System, Spain, while insulin measurement was done using an ELISA kit from Demeditec, Germany. The estimation of IR was performed using the homeostasis model assessment of insulin resistance (HOMA-IR), with IR calculated as fasting insulin multiplied by fasting glucose divided by 22.5. Serum TNF-α level was assessed by Human CBA Th1/Th2 cytokine kit (Cytometric Bead Array, BD Biosciences, San Jose, CA) according to the manufacturer's instructions.

4.5 | tatistical analysis

Arlequin software (version 3.1) and SNPstats were used to calculate allele frequency and genotypes http://bioinfo.iconcologia.net/SNPstats. SPSS software version 17 was used to analyze the data. ROC curve analysis was used to evaluate the accuracy of TNF gene expression, IR, fasting glucose, and fasting insulin levels. The best cutoff values were selected based on maximum sensitivity and specificity for prediction. The area under the curve criteria was used to qualify the accuracy. Two-sided tests were conducted with a significance level of 0.05. Independent t-tests, chi-square tests, One-way ANOVA, and Pearson correlation coefficient tests were used to compare groups and examine relationships between data.

5 | RESULTS

5.1 | General clinical information for acne patients and healthy controls

The clinical and general data pertaining to both the acne patients and healthy controls have been meticulously presented in Table 1. It is noteworthy that acne patients exhibited significantly higher levels of IR, fasting glucose, fasting insulin, serum TNF-α, and TNF-α folding change, when compared to healthy controls, thus implying a strong association between these factors and the presence of acne patients ($p = 0.001$). Specific regions of the TNF-α gene polymorphism, −863

TABLE 1 Clinical and laboratory characteristics for acne patients and healthy controls.

Variables	Acne patients (*N* = 87)	Healthy controls (*N* = 73)	*p*-Value
Age (mean ± SD)	26.88 ± 2.4	27.55 ± 4.1	0.530
Male (N/%)	35 (40.2%)	34 (46.5%)	0.420
Female (N/%)	52 (59.8%)	39 (53.5%)	0.541
Acne vulgaris severity			
® Mild	36 (41.3%)	NA	NA
® Moderate	27 (31%)	NA	NA
® Severe	24 (27.7%)	NA	NA
Fasting glucose (mmoL/L) (mean ± SD)	92.4 ± 1.9	78.7 ± 2.1	0.001
Fasting insulin (mlu/ L) (mean ± SD)	20 ± 2.4	13 ± 3.1	0.001
IR (HOMA-IR) (mean ± SD)	65 ± 5.9	41 ± 2.8	0.001
TNF-α (pg/mL) (mean ± SD)	11.8 ± 2.4	6.8 ± 2.3	0.001
TNF-α folding change ($2^{-\Delta\Delta c}$) (mean ± SD)	2.9 ± 0.7	1.09 ± 0.2	0.001

Abbreviations: %: percentage, SD, standard deviation; HOMA-IR, homeostasis model assessment of insulin resistance. NA, not applied.; IR, insulin resistance; *N*, number; TNF-α, tumor necrosis factor-alpha.

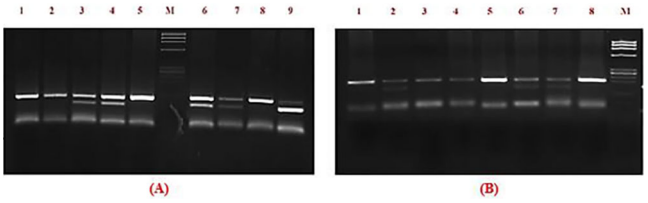

FIGURE 1 (A) Restriction enzyme analysis for −863 G > A (rs1800630) for TNF-α gene. Lanes 1,2,5, and 8 126 bp (C/C); lanes 3,4,6,7, and 9 126bp and 103bp (A/C); lane M, φx 174 markers. (B) Restriction enzyme analysis for −308 G > A (rs1800629) for TNF-α gene. Lanes 1,3,4,5, and 8 195bp (A/A); lanes 2,6, and 7 195bp and 173bp (G/A); lane M, φx 174 markers.

G > A (rs1800630), and −308 G > A (rs1800629) variants were subjected to amplification via PCR technique, as visually represented in the figures (Figure 1A,B).

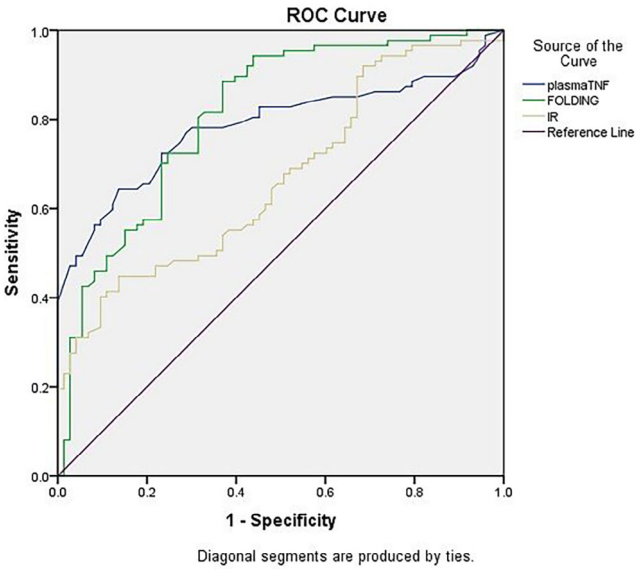

FIGURE 2 ROC curve analysis for TNF-α gene expression and biochemical parameters.

5.2 | Alternation in the expression of serum TNF-α and biochemical parameter in acne patients

The diagnostic accuracy of TNF-α gene expression in predicting disease was found to be excellent, with an area under the curve (AUC) value of 0.812. The confidence interval (CIs) for this prediction ranged from 74% to 87%, indicating a high level of certainty. Additionally, *p*-value associated with this prediction was 0.001, further supporting the significance of the findings. This diagnostic accuracy was achieved using a cutoff value of 1.3 for TNF-α gene expression. The sensitivity of this prediction was 88.5%, meaning that the test was able to correctly identify the presence of disease in 88.5% of cases. Furthermore, the specificity of the test was 63%, indicating its ability to correctly identify the absence of disease in 63% of cases.

In contrast, the diagnostic accuracy of serum TNF-α in predicting disease was deemed good, with an AUC value of 0.785 *p*-value associated with this prediction was also 0.001, suggesting its statistical significance. The cutoff value for serum TNF-α was determined to be 9.5 pg/mL. The sensitivity of this prediction was 64.4%, indicating that the test was able to correctly identify the presence of disease in 64.4% of cases. Additionally, the specificity of the test was 86.3%, signifying its ability to correctly identify the absence of disease in 86.3% of cases.

Moreover, it is important to note that the AUC for IR was found to be 0.668, with *p*-value of 0.001. These results suggested a moderate level of diagnostic accuracy for IR prediction. Figure 2 provides a visual representation of the data and further emphasizes the findings discussed above.

Overall, these findings highlighted the potential utility of TNF-α gene expression and serum TNF-α as diagnostic markers for disease prediction, as well as the relevance of IR in this context.

TABLE 2 Association of genetic variants in TNF-α gene in acne patients and healthy controls.

A) SNP	Model	Acne patients N (%)	Healthy controls N (%)	OR (95% CI)	*p*-Value
−863 G > A (rs1800630)					
	P(HWE)	0.33 [a]*	0.002*[b]		
	Co-dominant				
		42 (48.3%)	16 (21.9%)	1	
A/A		34 (39.1%)	26 (35.6%)	0.5 (0.23-1.08)	0.001*
A/G		11 (12.6%)	31 (42.5%)	0.14 (0.06-0.33)	
G/G					
	Dominant				
		42 (48.3%	16 (21.9%)		
A/A		45 (51.7%)	57 (78.1%)	1	5e-04*
G/A-G/G	Recessive			0.3 (0.15-0.60)	
		76 (87.4%)	42 (57.5%)		
A/A-G/A		11 (12.6%)	31 (42.5%)	1	0.001*
G/G				0.20 (0.09-0.43)	
−308 G > A (rs1800629)					
	P(HWE)	0.0095*[b]	0.0042*[b]	1	
	Co-dominant	26 (29.9%)	46 (63%)	3.4 (1.5-7.4)	1e-04*
A/A		31 (35.6%)	16 (21.9%)	4.8 (2.08-11.02)	
A/G		30 (34.5%)	11 (15.1%)		
G/G					
		26 (29.9%)	46 (63%)		
A/A	Dominant	61 (70.1%)	27 (37%)	1	0.001*
G/A-G/G				4 (2.03-7.7)	
		57 (65.5%)	62 (84.9%)		
A/A-G/A	Recessive	30 (34.5%)	11 (15.1%)	1	0.004**
G/G				2.9 (1.3-6.4)	
B) Allele frequency	**Acne patients N (%)**	**Healthy controls N (%)**	***p*-Value**		
−863 G > A (rs1800630)					
	56 (32%)	88 (60%)	0.001*		
G	118 (68%)	58 (40%)	0.004**		
A					
−308G > A (rs1800629)	91 (52%)	38 (26%)	0.001*		
G	83 (48%)	108 (74%)	0.003**		
A					

Abbreviations: CI, confidence interval; HWE, Hardy-Weinberg equilibrium; OR, odds ratio; rs, reference sequence.; SNP, single nucleotide polymorphism.
*Highly significant differences, $p \leq 0.01$.
**Mild significant differences $p \leq 0.05$.

5.3 | Genotypic and allelic frequencies for genetic variants in TNF-α gene

Hardy-Weinberg assumptions were met by two SNPs $p > 0.05$; Table 2A to find differences between acne patients and healthy controls, genotype and allele frequencies were computed and compared. The co-dominant model for −863 G > A (rs1800630) and −308 G > A (rs1800629) Different versions showed significant differences across the two groups ($p = 0.001$, and $p =$ 1e-04, respectively) (Table 2A).

To verify the connections of the two 863 G > A (rs1800630) and 308 G > A (rs1800629) variants with acne patients, dominant and recessive modeling for the uncommon allele were created and ORs and 95% CIs were generated. Table 2A,B show the genotypes and observed allele frequencies for acne sufferers and healthy controls.

TABLE 3 Comparison of biochemical parameters and TNF-α gene expression with −308 G > A (rs1800629) and −863 G > A (rs1800630) genotypes in acne patients.

(A)				
−308 G > A (rs1800629) variant	**A/ A (*N* = 26)**	**A/G (*N* = 31)**	**G/G (*N* = 30)**	***p*-Value**
IR (HOMA-IR) (mean ± SD)	64 ± 3.8	62 ± 5.9	74 ± 4.1	0.742
TNF-α (pg/mL) (mean ± SD)	14 ± 2.51	10.8 ± 3.5	8.7 ± 2.2	0.005**
TNF-α folding change $(2^{-\Delta\Delta ct})$ (mean ± SD)	3 ± 0.8	3.3 ± 0.9	1.7 ± 0.8	0.265
(B)				
−863 G > A (rs1800630) variant	**A/ A (*N* = 26)**	**A/G (*N* = 30)**	**G/G (*N* = 31)**	***p*-Value**
IR (HOMA-IR) (mean ± SD)	80.3 ± 6.5	68.4 ± 3.2	48.4 ± 2.9	0.002**
TNF-α (pg/mL) (mean ± SD)	14.3 ± 3.1	10.1 ± 2.9	10.2 ± 2.5	0.005**
TNF-α folding change $(2^{-\Delta\Delta ct})$ (mean ± SD)	4.3 ± 0.6	2.5 ± 0.8	2 ± 0.5	0.001*

Abbreviations: %: percentage, SD, standard deviation; HOMA-IR, homeostasis model assessment of insulin resistance. NA, not applied.; IR, insulin resistance; *N*, number; TNF-α, tumor necrosis factor-alpha.
*Highly significant differences, $p \leq 0.01$.
**Mild significant differences $p \leq 0.05$.

The assumptions of the Hardy-Weinberg equilibrium were satisfied by two variants with a significance level greater than 0.05, as shown in Table 2A. To identify discrepancies between acne patients and healthy controls, the frequencies of genotypes and alleles were calculated and compared. Notably, there were significant variations in genotype observed in the co-dominant model for the −863 G > A (rs1800630) and −308 G > A (rs1800629) variants between the two groups, with respective *p*-values of 0.001 and 1e-04 (Table 2A).

To further study the relationships of the two variations, 863 G > A and 308 G > A, with acne sufferers, recessive and dominant models for the rare allele were created. This was done to examine the differences and provide odds ratios (ORs) and 95% confidence intervals (CIs) to confirm the associations. Table 2A,B show the genotypes and estimated allele frequencies for acne sufferers and healthy controls.

5.4 | Compare the alternation in biochemical parameters and TNF-α gene expression with two variants of TNF gene

For −308 G > A (rs1800629) variants A/A, there is an association with elevated levels of serum TNF-α, with *p*-value of 0.005. Conversely, there is no significant variation observed in IR resistance and TNF-α folding change when considering −308 G > A genotypes as shown in Table 3A. However, in −863 G > A (rs1800630) A/A genotypes, it is noteworthy that higher levels of IR and TNF-α folding change were observed compared to A/G and G/G genotypes. In addition, these A/A genotypes exhibited a higher concentration of serum TNF-α in comparison, with statistically significant *p*-values of 0.002, 0.001, and 0.005 respectively, as demonstrated in Table 3B.

5.5 | Compare the alternation in biochemical parameters with two variants of TNF-α genotypes in different clinical variants

The analysis of biochemical parameters and the examination of different clinical types of acne patients revealed that there exists a significant disparity between the levels of serum TNF-α, IR, and TNF-α folding change among patients with severe acne in comparison to those affected by mild and moderate acne patients (*p*-values of 0.00, 0.001, and 0.005, respectively, as indicated in Table 4.

Severe acne patients who had the A/A genotype for −308 variants exhibited higher levels of IR, serum TNF-α, and TNF-α folding change, with *p*-values of 0.005, 0.001, and 0.001, respectively. The same results were observed in patients with the A/A genotype for −863 variant, with *p*-values of 0.003, 0.005, and 0.005, respectively (Table 5A). However, no significant variations were observed with mild and moderate acne patients when compared to different genotypes of −308 and −863 variants, as indicated by all the biochemical parameters presented in Table 5B,C.

5.6 | Correlation between serum TNF-α, TNF-α expression, and IR in severe acne patients

A remarkably strong and noteworthy positive correlation, that was found to be highly significant, emerged when examining the relationship between the change in TNF-α folding and the presence of IR. This correlation, denoted by the correlation coefficient (r) value of 0.598 and the *p*-value of 0.001, was established through a simple linear regression analysis (Figure 3).

Furthermore, we think it is important note that serum or TNF expression has correlation with IR. A correlation coefficient (r) value of 0.678 and *p*-value of 0.001 were obtained, signifying a strong and

TABLE 4 Comparison of biochemical parameters and variable clinical types of acne patients.

Parameters	Severe acne (*N* = 24)	Moderate acne (*N* = 27)	Mild acne (*N* = 36)	*p*-Value
IR (HOMA-IR) (mean ± SD)	82 ± 3.4	58 ± 4.6	55 ± 3.9	0.001*
TNF-α (pg/mL) (mean ± SD)	14.6 ± 2.3	11.2 ± 1.9	8.9 ± 2.2	0.001*
TNF-α folding change $(2^{-\Delta\Delta ct})$ (mean ± SD)	3.9 ± 0.5	2.8 ± 0.8	1.9 ± 0.6	0.005**

Abbreviations: %: percentage, SD, standard deviation; HOMA-IR, homeostasis model assessment of insulin resistance. NA, not applied.; IR, insulin resistance; *N*, number; TNF-α, tumor necrosis factor-alpha.
*Highly significant differences, $p \leq 0.01$.
**Mild significant differences $p \leq 0.05$.

TABLE 5 Comparison of biochemical parameters with different genotypes of two TNF-α variants according to clinical types of acne patients.

A. Severe acne patients				
−308 G > A (rs1800629) Variant	**A/ A (*N* = 6)**	**A/G (*N* = 9)**	**G/G (*N* = 9)**	***p*-Value**
IR (HOMA-IR) (mean ± SD)	80 ± 3.7	66 ± 3.9	64 ± 4.2	0.005**
TNF-α (pg/mL) (mean ± SD)	13.3 ± 1.7	5.3 ± 2.4	7.5 ± 2.3	0.001*
TNF-α folding change $(2^{-\Delta\Delta ct})$ (mean ± SD)	4.3 ± 0.4	2.3 ± 0.6	1.2 ± 0.3	0.001*
−863 G > A (rs1800630) variant	A/ A (*N* = 12)	A/G (*N* = 8)	G/G (*N* = 4)	*p*-Value
IR (HOMA-IR) (mean ± SD)	73 ± 2.6	60 ± 3.2	55 ± 1.7	0.003**
TNF-α (pg/mL) (mean ± SD)	14.3 ± 2.1	8.9 ± 3.4	7.9 ± 2.8	0.005**
TNF-α folding change $(2^{-\Delta\Delta ct})$ (mean ± SD)	3.2 ± 0.32	1.7 ± 0.42	1.6 ± 0.8	0.005**
B. Moderate acne patients				
−308 G > A (rs1800629) Variant	**A/ A (*N* = 7)**	**A/G (*N* = 9)**	**G/G (*N* = 11)**	***p*-Value**
IR (HOMA-IR) (mean ± SD)	57 ± 4.6	63 ± 6.4	75 ± 8.4	0.587
TNF-α (pg/mL) (mean ± SD)	9.8 ± 1.9	13.3 ± 2.1	10.4 ± 1.6	0.320
TNF-α folding change $(2^{-\Delta\Delta ct})$ (mean ± SD)	2.8 ± 0.8	3.6 ± 0.7	1.04 ± 0.7	0.103
−863 G > A (rs1800630) variant	A/ A (*N* = 10)	A/G (*N* = 12)	G/G (*N* = 5)	*p*-Value
IR (HOMA-IR) (mean ± SD)	52 ± 5.9	82 ± 2.2	58 ± 3.1	0.167
TNF-α (pg/mL) (mean ± SD)	12 ± 2.2	11 ± 3.3	9 ± 2.9	0.532
TNF-α folding change $(2^{-\Delta\Delta ct})$ (mean ± SD)	3.2 ± 0.5	2.7 ± 0.7	1.3 ± 0.6	0.354
C. Mild acne patients				
−308 G > A (rs1800629) Variant	**A/ A (*N* = 13)**	**A/G (*N* = 13)**	**G/G (*N* = 10)**	***p*-Value**
IR (HOMA-IR) (mean ± SD)	80 ± 3.8	56 ± 2.8	41 ± 4.7	0.449
TNF-α (pg/mL) (mean ± SD)	15 ± 3.6	15 ± 2.7	14 ± 4.1	0.998
TNF-α folding change $(2^{-\Delta\Delta ct})$ (mean ± SD)	2.7 ± 0.9	3.2 ± 0.87	3.9 ± 0.77	0.144
−863 G > A (rs1800630) variant	A/ A (*N* = 20)	A/G (*N* = 14)	G/G (*N* = 2)	*p*-Value
IR (HOMA-IR) (mean ± SD)	72 ± 3.2	46 ± 2.7	31 ± 3.9	0.345
TNF-α (pg/mL) (mean ± SD)	14 ± 1.9	14 ± 2.8	16 ± 2.7	0.921
TNF-α folding change $(2^{-\Delta\Delta ct})$ (mean ± SD)	2.8 ± 0.9	4.1 ± 0.6	2.9 ± 0.61	0.444

Abbreviations: %: percentage, SD, standard deviation; HOMA-IR, homeostasis model assessment of insulin resistance. NA, not applied.; IR, insulin resistance; *N*, number; TNF-α, tumor necrosis factor-alpha.
*Highly significant differences, $p \leq 0.01$.
**Mild significant differences $p \leq 0.05$.

statistically significant correlation (Figure 4). Further reinforces this finding, demonstrating the consistency of the relationship between these variables. Thus, it can be concluded that both the change in TNF-α folding and serum TNF-α levels are positively correlated with the presence of IR, as indicated by the robust statistical analysis and visual representations.

6 | DISCUSSION

The etiopathogenesis of acne is multifactorial. Cutibacterium acnes colonization, inflammation, and estrogen-dependent sebogenesis are the four main components implicated in the pathophysiology of acne.[10] The genotypes of glutathione S-transferases (GSTs) and TP53 play

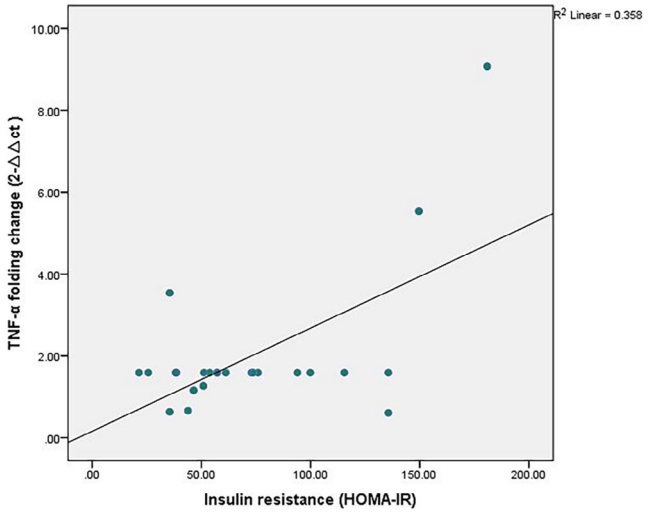

FIGURE 3 Correlation between TNF-α gene folding and IR.

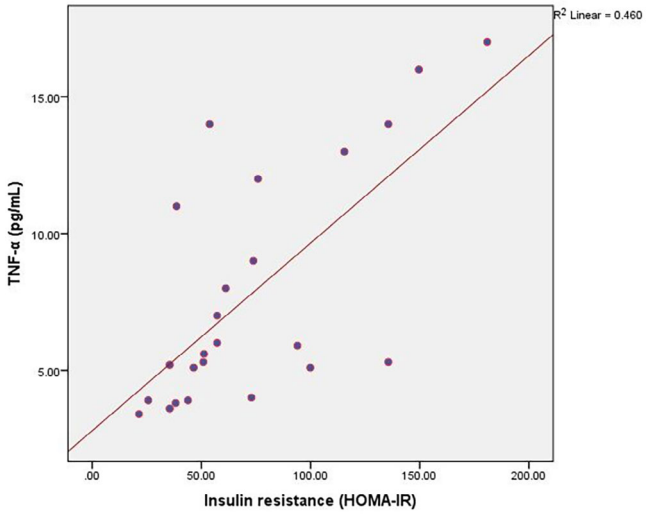

FIGURE 4 Correlation between serums TNF-α and IR.

a role in protecting against oxidative stress and may influence AV progression.[11] Also, patients diagnosed with TCAC and CAGG had a higher prevalence than healthy patients with TAAC and CCAC. The linkage disequilibrium between rs433235 A > G and IVS5.59 C > A has been identified. Furthermore, there seems to be a strong linkage disequilibrium between rs3747531 C > G and rs433235 A > G.[12]

Pro-inflammatory cytokines like TNF-α are key factors in these events. The TNF-α gene's SNPs have been linked to a higher risk of developing chronic inflammatory disorders.[13]

High IL-12 levels can cause chronic inflammation and aggravate IR.[14] Also, TNF-α first recognized to be involved in the pathogenesis of IR and glucose-related abnormalities. IR's role in the pathophysiology of acne is yet to be clear, though elevated blood sugar levels stimulate insulin synthesis and secretion, which in turn stimulate the production of androgens by the adrenal glands and ovaries.[15] Additionally, it has the ability to decrease the blood level of sex hormone-binding globulin (SHBG), which can significantly increase androgen activity and promote the development of AV.[16]

This study was conducted to investigate the significance of two genetic variants in the promoter region of the TNF-α gene and their association with IR in acne patients to understand how these variants contribute to AV and its associated IR.

This study showed that IR, serum TNF-α, and TNF-α folding changes were associated with AV, especially severe grades of acne. IR is the inability of insulin to provide glucose transport into the tissue despite normal or elevated levels of insulin; as a consequence, there is hyperglycemia and hyperinsulinemia.[15]

As we see in our study, patients with AV had higher fasting glucose and fasting glucose levels. In accordance with our study, a considerable link has been observed by Nagpal et al.[17], between the HOMA-IR value and the onset of AV. However there has not been any evidence of a statistically significant relationship between the severity of AV, the metabolic syndrome, and the HOMA-IR value;[18] also, a noteworthy association between the HOMA-IR and the acne score was observed in the Del Prete et al., study, which involved male acne patients.[18] Kartal et al., have reported that IR is an independent risk factor for acne apart from hyperandrogenemia.[19] A considerably higher HOMA value (HOMA = 2.87) was reported to Emiroglu et al., for both male and female acne patients; the value indicated that the IR had already begun to develop.[20]

Nevertheless, some research has found no link between the onset of AV and IR. Balta et al., have reported no statistically significant variations in the serum concentrations of fasting insulin, fasting blood glucose, and HOMA-IR value between the control group and patients with post-adolescent acne.[21] The different results here may be due to the low glycemic index optimum diet that was started for both the acne patients and healthy controls at least two weeks before the HOMA-IR test, and the sample was only on post-adolescent acne.

No correlation has been shown by Cetinözman et al., between hyperandrogenism, or IR, and severe acne. The difference in the results in this study may be due to the small sample size, which was about 26 acne patients and 21 healthy controls, and they were all females with post-adolescent severe acne.[22]

The purpose of this study is to investigate the significance of the −863 G > A (rs1800630) and −308 G > A (rs1800629), variants in the modulation of TNF-α gene expression in AV patients. We found that there was a significant variation of the −863 G > A (rs1800630) and −308 G > A (rs1800629) variants in co-dominant, dominant, and recessive models between AV patients and healthy controls, with the A/A genotype being more prevalent in AV patients than healthy controls. Also, we found that the allelic frequency of A is higher in AV patients with −863 G > A (rs1800630) polymorphism, while the G allele is more prevalent in AV patients with −308 G > A (rs1800629) polymorphism.

The A/A genotype of −863 G > A (rs1800630) variant is associated with a significant increase in serum TNF-α, and the A/A genotype of −308 G > A (rs1800629) variant is associated with a significant increase in IR, serum TNF-α, and TNF-α folding change, and they are elevated in severe AV patients but not in mild or moderate AV patients. In agreement with our study, research done by Li et al. showed that

TNF-α −308 G > A genotype AA may play a role in the etiology of AV. Therefore, identifying the TNF-α −308 G > A polymorphism could be a useful biomarker for AV patient's early identification.[23]

Szabó et al. found an increase in the minor −308 A allele, especially in female AV sufferers, with increased minor A allele frequencies in AV patients with more severe cases of inflammatory AV.[13] On the other hand, no connection has been shown between any alleles of −1031 T > C, −863 C > A, and −238 G > A polymorphisms and AV. Also, a study done by Baz et al., on a Turkish population composed of 113 AV patients and 114 healthy controls. When comparing AV patients to healthy controls, they discovered that the prevalence of the TNFA −308 GA genotype was statistically considerably higher in AV patients. This was similar to our study, but in reverse, there was no correlation between the severity of AV patients and TNFA genotypes, and this may be due to different ethnic groups of AV patients. They also explained this by considering the possibility that other variables, such as the environment and other genetic components, might impact AV severity more easily.[24]

In the research performed by Al-Shobaili et al. significant differences in the frequency of genotypic variations of the TNF-α polymorphism were detected between AV patients and healthy controls in Saudi Arabia. The frequency of TNF-α −308 GG and AA homozygosity genotypes was much greater in AV sufferers than in healthy controls, and these findings are consistent with ours. In comparison to their respective healthy controls and female cases, male cases displayed higher frequencies of the high-production allele genotypes TNF-α −308 AA and GA, while female cases displayed more homozygosity for the low-production genotype GG. This could lead them to hypothesize that TNF-α has a modulatory rather than a stimulatory role in the pathophysiology of AV. Among cases with different genders, it may also be associated with other interactive characteristics, most likely hormonal in nature, but this speculation cannot be confirmed because of the small sample size, especially the male patients.[25]

Three SNPs (−376, −308, and −238) of the TNF gene were examined in a study by Grech et al., in a white population with light skin. According to the study, male patients with the GAG haplotype had an earlier onset of AV than those with the GGG haplotype, which was linked to an increased risk of AV at a later age.[26] Also, in agreement with our results, there was a correlation found between the AA genotypes of −308 and −238 with a higher likelihood of acquiring AV patients in Pakistani with considerably greater frequency of the mutant A allele at positions −308 and −238 in comparison to the healthy controls. Additionally, a correlation was seen between the genotypes of variants −308 and −238 with the severity of AV.[27]

Conversely, Sobjanek et al. found no correlation between Polish AV patients and the TNF- α gene promoter polymorphisms at positions −238 and −308 which may be a result of different ethnic groups from our patients.[28]

Analysis was done on 441 Pakistani AV patients (287 females and 154 males) and 303 healthy controls (111 females and 192 males) for the polymorphism at −863 C/A location. The examination of −863 G/A genotypes and allele frequency distribution revealed that this polymorphism was associated with AV in males based on the dominant model of association penetrance and in females based on the general model.[4]

The comparison of serum TNF-α in acne patients with varying degrees of acne severity and acne types has suggested that studied TNF-α polymorphisms are not associated with acne severity and acne type. This is in contrast with our results; the reason for these dissimilarities is not clear but might reflect differences in the linkage groups that are associated with the TNF-α −863 SNP in the two-studied populations.[29]

7 | CONCLUSION

IR, TNF-α, and TNF-α folding change are associated with AV especially severe grades of acne. The −863 G > A (rs1800630) and −308 G > A (rs1800629) variants polymorphism might have a modulatory effect on the TNF-α gene expression and in consequence, serum TNF-α levels which have a prominent role in the pathogenesis of AV and has association with IR in acne patients. But further studies should be carried out with more sample size and same and different ethnic groups to confirm these effects.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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