Original Article

Reappraisal of Acne Vulgaris Pathogenesis: The Role of Tissue Inhibitor of Metalloproteinase-2 (TIMP-2)

Wafaa Shehata[1], Sally Elhefnawy[2], Mohamed Basha[1], Maha Abo El-Khier[1], Sherin Sobhy Elnaidany[3]

[1]Department of Dermatology, Andrology and STDs, Menoufia University Faculty of Medicine, Menoufia, Egypt
[2]Department of Basic Medical Science, Alryada University for Science and Technology (RST) Faculty of Dentistry, Sadat City, Egypt
[3]Department of Medical Biochemistry and Molecular Biology, Menoufia University Faculty of Medicine, Shebin El-Kom, Egypt

Abstract

Aim: Acne vulgaris is a persistent inflammatory condition affecting the pilosebaceous unit. Tissue inhibitor of metalloproteinase-2 (TIMP-2) is an endogenous inhibitor of matrix metalloproteinase-2 (MMP-2) that controls MMP-2 proteolytic activity. This study was designed to evaluate serum and gene expression levels of TIMP-2 in patients with acne vulgaris compared with healthy controls, and to correlate these results with available clinical data.

Materials and Methods: This case-control study included 83 cases of acne vulgaris and 83 normal controls. Disease severity was estimated by the Global Acne Grading System (GAGS). Real-time polymerase chain reaction was used to determine *TIMP-2* gene expression, and serum TIMP-2 levels were assessed by enzyme-linked immunosorbent assay.

Results: Comparison between cases and controls showed statistically significant differences in mean serum levels and TIMP-2 expression levels, with levels higher in cases than in controls ($P < 0.001$ for both). Significant positive correlations were observed between mean serum TIMP-2 levels and the GAGS score, NAST classification, and *TIMP-2* gene expression ($P < 0.001$ for all). Significant positive correlations were observed between *TIMP-2* gene expression and both the GAGS score and the NAST classification ($P < 0.001$ for both).

Conclusion: Serum TIMP-2 levels and *TIMP-2* gene expression are significantly elevated in patients with acne vulgaris and positively correlate with disease severity. These findings suggest that TIMP-2 may play a role in acne pathogenesis and could serve as a biomarker of disease activity.

Keywords: Acne vulgaris, tissue inhibitor of metalloproteinase-2, matrix metalloproteinase-2, polymerase chain reaction, enzyme-linked immunosorbent assay

Introduction

Acne vulgaris ranks as one of the most prevalent dermatological problems affecting the pilosebaceous unit, which is made up of the hair follicle, shaft, and sebaceous gland.[1] Acne vulgaris typically begins in adolescence, reaches a peak prevalence of about 95% between the ages of 14 and 19 and often resolves by the middle of one's 20s.[2]

Acne vulgaris commonly presents as pimples, nodules, pustules, and cysts, and these lesions can often result in pigmentation and scarring. Patients with severe acne may have permanently disfiguring scars, which may negatively affect their appearance and their psychological health.[3]

Submission: 05-Apr-2026
Acceptance: 04-Jul-2026 **Web Publication:** 02-Sept-2026

Access this article online

Quick Response Code:

Website: www.turkjdermatol.com

DOI: 10.4274/tjd.galenos.2026.94546

Address for correspondence: Sherin Sobhy Elnaidany, Department of Medical Biochemistry and Molecular Biology, Menoufia University Faculty of Medicine, Shebin El-Kom, Egypt
Email: sherinsobhy@ymail.com
ORCID ID: 0000-0002-2129-0508

How to cite this article: Shehata W, Elhefnawy S, Basha M, El-Khier MA, Elnaidany SS. Reappraisal of acne vulgaris pathogenesis: the role of tissue inhibitor of metalloproteinase-2 (TIMP-2). Turk J Dermatol. 2026;20(3):119-130.

Acne has a complex, heterogeneous pathophysiology. The main pathogenic factors include androgen stimulation of sebaceous secretion, increased sebum secretion, and follicular hyperkeratinisation. It has been suggested that microbial colonization by *Cutibacterium acnes (C. acnes)* increases perifollicular inflammation, which is a crucial early step in the development of acne vulgaris.[4]

Many matrix metalloproteinases (MMPs), including MMP-1, MMP-2, MMP-3, MMP-9, and MMP-13, can be stimulated by *C. acnes*.[5] MMPs' primary function is to degrade all components of the extracellular matrix. However, they also play a part in inflammation, cell development, proliferation, and remodeling, as well as influencing the production and release of cytokines and chemokines.[6]

Tissue inhibitor of metalloproteinase-2 (TIMP-2) is an endogenous inhibitor of MMP-2, regulating its proteolytic activity.[7] TIMP-2, located on the long arm of chromosome 17, locus number (25), has been shown to regulate the proteolytic activity of MMP-2 through the enzyme's production of a 1:1 stoichiometric suppressive complex.[8]

TIMP-2 and MMP-2 interact via a mechanism more complex than a simple inhibitor-substrate interaction. The relation between TIMP-2 and MMP-2 is concentration-dependent. At low concentrations, TIMP-2 can bind to membrane type-1 MMP and the carboxyl-terminal hemopexin-like domain of MMP-2, activating pro-MMP-2 and promoting its maturation. At high concentrations, TIMP-2 can bind MMP-2 via its N-terminal domain, thereby inhibiting MMP-2 activity.[9]

The study aims to assess TIMP-2 messenger ribonucleic acid (mRNA) expression and serum TIMP-2 levels in patients with acne vulgaris of varying grades and to correlate these findings with available clinical data.

Materials and Methods

In this case-control study, 83 patients with acne vulgaris served as the case group, and 83 age- and gender-matched healthy subjects served as the control group.

Inclusion Standards

The following individuals were recruited to participate in the study after obtaining informed consent: patients presenting with acne vulgaris who had not received treatment for three months, had no history of skin resurfacing, and had not used oral isotretinoin in the previous six months.

Exclusion Standards

Exclusion criteria are as follows: patients with a history of diabetes mellitus; patients who are obese; females with polycystic ovary syndrome; patients with chronic or acute hepatitis; patients with liver cirrhosis; patients with benign or malignant tumours; patients with inflammatory bowel disease; patients with chronic pancreatitis; patients with cardiovascular diseases, autoimmune disorders, malignancy, or asthma; patients receiving drugs that cause acneiform eruptions; patients with a known history of lipid metabolic disorder or those taking drugs that affect lipid metabolism; subjects with acne conglobata, acne fulminans, secondary acne (chloracne, drug-induced acne, etc.), or any other cutaneous or fibrotic lesions.

Sample Size Calculation

A study proposing to evaluate the effect of TIMP-2 mRNA expression on acne vulgaris of varying severity, compared with healthy controls. A previous study showed that the distribution of the GC genotype for the TIMP-2 (-418 G/C) polymorphism differed between the patient and control groups, 14.8% vs. 33.3%, respectively.[10] Thus, the sample size for the current study, to detect a significant effect at $P < 0.05$ with 80% power was calculated using the following formula:

$$p = \frac{p_1 + rp_2}{1+r}$$

$$n \geq \frac{\left[Z_{1-\alpha/2}\sqrt{(r+1)p(1-p)} + Z_{1-\beta}\sqrt{rp_1(1-p_1)+p_2(1-p_2)}\right]^2}{r(p_2-p_1)^2}$$

In the above formula, Z1-α/2 = 1.96; β = 0.2; r = 1. So, n = 83. Thus, at least 166 individuals should be recruited for the study, with at least 83 in each group. n is the minimum sample size for the group. Z1-α/2 denotes the standardized value corresponding to the specified confidence level. At 95% confidence interval (CI), it is 1.96, and at 99% CI (1% type I error), it is 2.58. The sample consisted of 166 patients.

Consent to Participate and Ethics

Prior to the initiation of the study, ethical approval was obtained from the Research Ethics Committee, Institutional Review Board, Faculty of Medicine, Menoufia University (approval no: DERMA 27; approval date: 27 October 2022). The study was conducted in accordance with the principles of the Declaration of Helsinki. Cases were consecutively recruited from the Outpatient Clinic of Dermatology, Andrology and STDs at Menoufia University Hospitals. Each participant

provided written informed consent before the start of the study, which was approved by the Local Ethical Research Committee of Menoufia Faculty of Medicine. One of the patients' parents provided consent for the patients under the age of eighteen. Every enrolled patient underwent thorough history-taking and a detailed dermatological examination. Along with other clinical information, the following were gathered: onset, progression, lesion location, aggravating factors (food, stress, pregnancy, menstruation, sun exposure, etc.), and family history.

Clinical evaluation of the enrolled patients was performed in accordance with Nast et al.,[11] who assessed acne vulgaris as follows: the microcomedone gave rise to non-inflammatory lesions, including both closed (whiteheads) and open (blackheads) comedones. Inflammatory lesions were either deep or superficial. The terms papules and pustules refer to superficial inflammatory lesions measuring ≤ 5 mm in diameter. In more severe cases, lesions could progress to nodules or deep pustules.

Evaluation of Acne Vulgaris Severity

Acne vulgaris was categorized according to the method described by Tan.[12] Mild acne was defined as the presence of < 20 comedones, < 15 inflammatory lesions, or < 30 total lesions. Moderate acne was defined as 20–100 comedones, 15–50 inflammatory lesions, or a total lesion count of 30–125. Severe acne vulgaris was defined as the presence of more than five cysts, more than 100 comedones, more than 50 inflammatory lesions, or a total lesion count of more than 125.

Acne Vulgaris Lesion Scoring

The severity score is the result of adding up the six regional subscores, which are obtained by multiplying the factor for each region (two seconds for the forehead and cheeks, one second for the nose and chin, and three seconds for the chest and back) by the most severely weighted lesion within that region (1 for ≥ 1 comedone, 2 for ≥ 1 papule, 3 for ≥ 1 pustule, and 4 for ≥ 1 nodule). Global Acne Grading System (GAGS) scoring methodology was based on a quantitative scoring system.[13]

Sampling and Preparation

A total of 5 mL of venous blood was collected from each participant and divided into two aliquots. Accordingly, 3 mL was placed in a standard tube, allowed to clot for 30 minutes at room temperature, and then centrifuged for 10 minutes at 4,000 revolutions per minute. The serum was separated and stored for the determination of TIMP-2 (ng/mL) by enzyme-linked immunosorbent assay (ELISA). Additionally, 2 mL of blood was collected into a Vacutainer tube containing EDTA for mRNA extraction, and the extract was stored at -80 °C until the RT-PCR step.

Protein and Gene Analysis

Human serum TIMP-2 concentrations (ng/mL) were measured using the SUN RED (ST) ELISA kit (Shanghai). Quantitative real-time PCR was performed using the *TIMP-2* gene as the target and GAPDH as the housekeeping (reference) gene. We used the miRNeasy Mini preparations (catalogue number 217004, Qiagen, Germany) for whole RNA extraction, combining silica-membrane purification with phenol/guanidine-based lysis. A Thermo Scientific (USA) NanoDrop 2000 spectrophotometer, set to measure absorbance at 260 and 280 nm, was used to determine the RNA concentration. A high-capacity cDNA synthesis kit with an inhibitor (Thermo Fisher Scientific, Applied Biosystems, USA) was used for reverse transcription (RT) of RNA into cDNA. RT was conducted using 10 µL of RNA in a 20 µL reaction volume, and 2 µL of RT random primers, 2 µL of dNTP mix, 1 µL of RNase inhibitor, and 1 µL of reverse transcriptase MultiScribe, and 2 µL nuclease free water. Incubation was done in a 2720 thermal cycler (Applied Biosystems, Singapore), single cycle at 25 °C for 5 minutes, for 60 minutes at 42 °C, and 70 °C for 5 minutes.

The expression levels of TIMP2 and GAPDH were quantified by qRT-PCR. The TIMP-2 forward primer was 5′ ACCCTCTGTGACTTCATCGTGC 3′, and the TIMP-2 reverse primer was 5′ GGAGATGTAGCACGGGATCATG 3′. The GAPDH forward primer was 5′CTCTGCTCCTCCTGTTCGAC3′, and the GAPDH reverse primer was 5′ TTAAAAGCAGCCCTGGTGAC3′. Each reaction for each gene was performed in a final volume of 20 mL, 5 µL of nuclease-free water was used. 3 µL of cDNA mix. 10 µL mL SYBR Green 2x QuantiTect PCRMaster. One mL forward primer, 1 mL reverse primer. The mix was incubated at 94 °C for 3 min, followed by 60 cycles; denaturation at 94 °C for 30 s, for 40 s for annealing at 55 °C and extension at 72 °C for 31 s.

All experimental conditions were carefully controlled, and PCR amplification efficiency was verified. Comparable amplification efficiencies for the target and housekeeping genes were assumed. The melting curve was produced (Figures 1 and 2). Higher Ct values were associated with

***TIMP-2* expression = $2^{-\Delta\Delta Ct}$**	
ΔΔCt =	**ΔCt Acne vulgaris sample-ΔCt control sample**
ΔCt acne vulgaris sample =	Ct (*TIMP-2* mRNA)–Ct (*GAPDH* mRNA)
ΔCt control =	Ct (*TIMP-2* mRNA)–Ct (*GAPDH* mRNA)

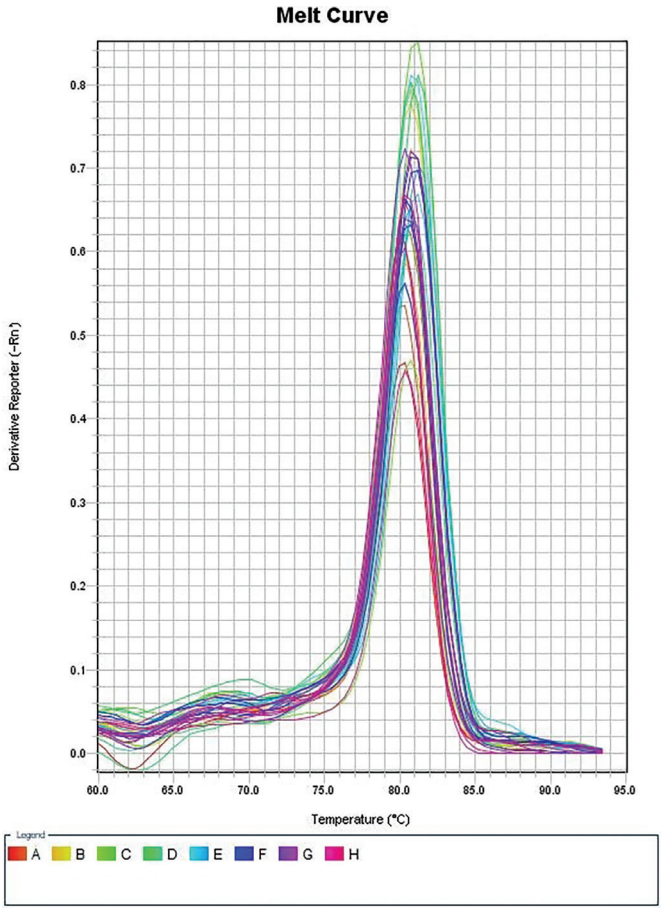

Figure 1. Melting curve of GAPDH

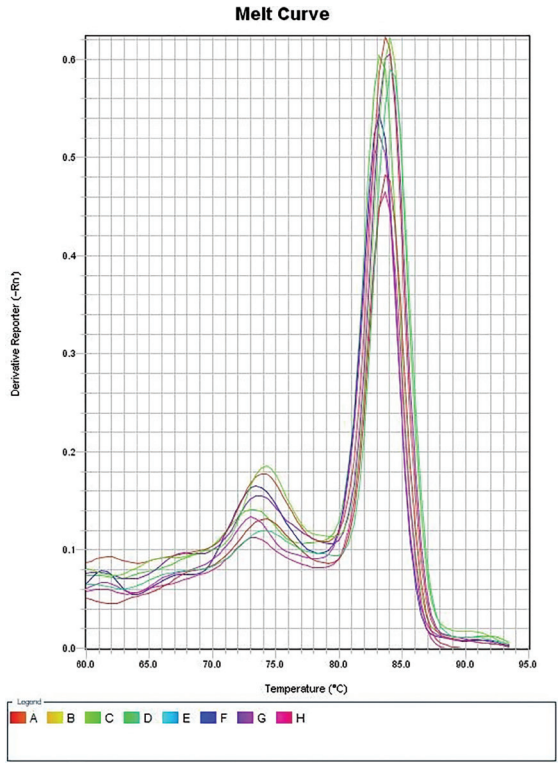

Figure 2. Melting curve of TIMP-2
TIMP-2: Tissue inhibitor of metalloproteinase-2

lower RNA levels. The relative quantification values for each unique gene were determined using the $2^{-\Delta\Delta Ct}$ formulation[14] in the following manner:

TIMP-2 expression = $2^{-\Delta\Delta Ct}$	
ΔΔCt =	ΔCt Acne vulgaris sample - ΔCt Control sample
ΔCt acne vulgaris sample =	Ct (*TIMP-2* mRNA) – Ct (*GAPDH* mRNA)
ΔCt control =	Ct (*TIMP-2* mRNA) – Ct (*GAPDH* mRNA)

Statistical Analysis

For the statistical analysis, an IBM personal computer running the statistical programs Epi Info 2000 (Centers for Disease Control and Prevention, Atlanta, GA, USA) and SPSS version 20.0 (IBM Corp., Armonk, NY, USA) was used. Descriptive statistics were performed as follows: categorical data were presented as numerical counts (N) and percentages (%), and quantitative data were presented as mean (X), range, and standard deviation (SD). Among the analytical procedures employed were the Spearman correlation coefficient test (r), the Mann–Whitney U test (U), the chi-square test (X^2), and the Kruskal–Wallis test (K). At $P \leq 0.05$, the significance threshold was applied.

Results

This case-control study included 35 males (42.2%) and 48 females (57.8%). Ages ranged from 16 to 28 years, with 20.82 ± 2.69 years for the mean ± SD value. Controls consisted of 35 (42.2%) males and 48 (57.8%) females, with ages ranging from 16 to 28 years. Mean ± SD value was 20.82 ± 2.69 years.

Clinical information for the studied cases of acne vulgaris is presented in Table 1.

Appraisal of Mean Serum TIMP-2 Level and Mean Expression Level of *TIMP-2* Gene Between Patients and Control Subjects

Upon studying the mean serum level of TIMP-2 and the mean expression level of the *TIMP-2* gene, there were statistically significant differences, both being higher in cases (63.58 ± 29.80 ng/mL) than in control subjects (22.36 ± 7.88 ng/mL) ($P < 0.001$ for both) (Table 2).

Relationship Between the Mean Serum Level of TIMP-2 (ng/mL) and Clinical Information of the Studied Patients

A significant association between the mean serum TIMP-2 level (ng/mL) and aggravating factors such as sun exposure (*P*

= 0.018) was observed. Additionally, significant relationships were found between the mean serum TIMP-2 level (ng/mL) and acne lesions on the back ($P < 0.001$) and chest ($P = 0.001$), NAST classification ($P < 0.001$), disease severity ($P = 0.01$), and GAGS grade ($P < 0.001$) (Table 3).

Table 1. Clinical facts of studied patients (n = 83)

Studied variables		n	%
Onset	Gradual	56	67.5
	Acute	27	32.5
Disease course	Progressive	49	59.0
	Stationary	34	41.0
Duration/years	Range	1.0–6.0	
	$\bar{x} \pm SD$	3.14 ± 1.34	
Family history	Positive	44	53.0
	Negative	39	47.0
Site	Face	83	100.0
	Back	63	75.9
	Chest	22	26.5
Aggravating factor	No	25	30.1
	Yes[#]	58	69.9
	Spicy food	21	25.3
	Sun	35	42.2
	Stress	52	62.7
	Menses	36	43.3
NAST classification	Non-inflammatory	36	43.4
	Inflammatory	47	56.6
Disease severity	Mild	27	32.5
	Moderate	28	33.7
	Severe	28	33.7
GAGS grade	Mild	36	43.4
	Moderate	24	28.9
	Severe	23	27.7
GAGS score	Min–max	6.0–37.0	
	X ± SD	18.89 ± 10.13	
	Median (IQR)	21.0 (9.0–31.0)	

X: Mean, n: Number, %: Percentage, [#]: More than one answer, SD: Standard deviation, IQR: Interquartile range, GAGS: Global Acne Grading System

Correlation Between Serum TIMP-2 (ng/mL) and Age of Cases in Years, Disease Duration in Years, GAGS Score, and *TIMP-2* Gene Expression

There were statistically significant positive correlations between mean serum TIMP-2 level (ng/mL) and duration of disease in years (r = 0.265, $P = 0.015$), GAGS score (r = *0.852), P < 0.001,* and *TIMP-2* gene expression level (r = 0.695), $P < 0.001$ (Figure 3a-c).

Relationship Between Mean *TIMP-2* Gene Expression Level and Clinical Information of the Studied Patients

Significant associations were observed between the mean expression level of the *TIMP-2* gene and (1) acne lesions on the chest ($P = 0.031$), (2) NAST classification ($P < 0.001$), and (3) GAGS grade ($P < 0.001$) (Table 4).

Correlation Between *TIMP-2* Gene Expression and Age of Cases in Years, Disease Duration in Years, and GAGS Score

Significant positive correlations were observed between *TIMP-2* gene expression and duration of disease in years (r = 0.286, $P = 0.009$), and between *TIMP-2* gene expression and GAGS score (r = 0.672, $P < 0.001$) (Figure 4a and b respectively).

ROC Curve for Serum TIMP-2 (ng/mL) and Expression of the *TIMP-2* Gene to Distinguish Cases from Control Subjects

For serum TIMP-2 (ng/mL), the receiver operating characteristic (ROC) curve showed a cut-off value of ≥ 31, with a sensitivity of 92.77%, specificity of 80.72%, and an area under the curve (AUC) of 0.969, which was statistically significant ($P < 0.001$). In comparison, *TIMP-2* gene expression at a cut-off value of ≥ 1.5 had a sensitivity of 93.98% and specificity of 100.0%. The AUC was 0.996 (*P*

Table 2. Evaluation of serum TIMP-2 (ng/mL) and *TIMP-2* gene expression levels between cases and controls

	Cases (n = 83)	Controls (n = 83)	U	*P*
Serum TIMP-2 (ng/mL)				
Min–max	30.0–120.0	7.50–34.0	213.000*	< 0.001*
Mean ± SD	63.58 ± 29.80	22.36 ± 7.88		
Median (IQR)	47.0 (43.0–88.0)	22.0 (17.0–31.0)		
***TIMP-2* gene**				
Min–max	1.35–6.80	0.52–1.50	25.000*	< 0.001*
Mean ± SD	3.74 ± 1.51	1.02 ± 0.23		
Median (IQR)	3.20 (2.80–4.0)	1.0 (0.86–1.17)		

SD: Standard deviation, IQR: Interquartile range, U: Mann–Whitney test, *P*: For comparison between diverse groups, TIMP-2: Tissue inhibitor of metalloproteinase-2, Min-max: Minimum-maximum
*: $P \leq 0.05$ is the level of significance

Table 3. Relationship between serum TIMP-2 (ng/mL) and clinical data of the studied cases (n = 83)

	n	Serum TIMP-2 (ng/mL)		Test of sig.	*P*
		X ± SD	Median (min–max)		
Sex					
Male	**35**	63.84 ± 29.19	47.0 (31.0–112.0)	U = 815.500	0.821
Female	**48**	63.39 ± 30.54	47.0 (30.0–120.0)		
Onset					
Gradual	**56**	64.76 ± 30.62	47.0 (30.0–120.0)	U = 748.0	0.938
Acute	**27**	61.15 ± 28.42	47.0 (31.0–112.0)		
Course					
Stationary	**34**	59.87 ± 30.79	44.50 (30.0–112.0)	U = 673.50	0.139
Progressive	**49**	66.16 ± 29.13	60.0 (31.0–20.0)		
Aggravating factor					
No	**25**	53.98 ± 25.58	44.0 (31.0–110.0)	U = 545.50	0.074
Yes[#]	**58**	67.72 ± 30.73	53.0 (30.0–120.0)		
Spicy food	**21**	59.66 ± 30.02	45.0 (31.0–120.0)	U = 581.50	0.466
Sun	**35**	73.26 ± 32.03	87.0 (30.0–120.0)	U = 585.0*	0.018*
Stress	**52**	67.98 ± 30.56	53.0 (30.0–120.0)	U = 606.0	0.059
Menses'	36	68.28 ± 30.32	59.50 (30.0–120.0)	U=707.50	0.202
Family history					
No	**39**	69.18 ± 29.96	60.0 (30.0–120.0)	U = 669.0	0.084
Yes	**44**	58.63 ± 29.10	45.0 (31.0–120.0)		
Site[#]					
Face	**83**	63.58 ± 29.80	47.0 (30.0–120.0)	–	–
Back	**63**	70.12 ± 29.93	60.0 (30.0–120.0)	U = 231.0*	< 0.001*
Chest	**22**	78.27 ± 27.30	86.0 (45.0–120.0)	U = 358.50*	0.001*
NAST classification					
Non-inflammatory	**36**	38.55 ± 5.89	39.50 (30.0–47.0)	U = 24.0*	< 0.001*
Inflammatory	**47**	82.76 ± 26.28	88.0 (45.0–120.0)		
Disease severity					
Mild	**27**	54.67 ± 30.36	43.0 (31.0–120.0)	K = 9.247*	0.010*
Moderate	**28**	67.16 ± 32.46	48.25[a] (30.0–112.0)		
Severe	**28**	68.61 ± 25.24	60.0[a] (43.0–120.0)		
GAGS grade					
Mild	**36**	38.55 ± 5.89	39.50 (30.0–47.0)	K = 64.475*	< 0.001*
Moderate	**24**	66.48 ± 18.01	60.0[a] (45.0–90.0)		
Severe	**23**	99.74 ± 22.68	110.0[ab] (47.0–120.0)		

SD: Standard deviation, GAGS: Global Acne Grading System, U: Mann–Whitney test, TIMP-2: Tissue inhibitor of metalloproteinase-2, K: Kruskal–Wallis test, *P*: For comparison between diverse groups using a post-hoc test (Dunn's test with Bonferroni adjustment for multiple comparisons) n: Number, *P*: For comparison between diverse groups
*: $P \leq 0.05$ is the level of significance, X: Mean
[a]: Significance with mild, [b]: Significance with moderate

< 0.001), indicating a significant ability to distinguish acne vulgaris cases from controls (Figure 5a).

ROC Curve for Serum TIMP-2 (ng/mL) and *TIMP-2* Gene Expression to Discriminate Disease Severity

A ROC curve was applied to serum TIMP-2 (ng/mL) and *TIMP-2* gene expression to determine their sensitivity and specificity for stratifying disease severity. Serum TIMP-2 at a cut-off value of ≥ 47 had a sensitivity of 60.71% and specificity of 61.82%. The AUC was 0.645, with a statistically significant result ($P = 0.031$). In comparison, *TIMP-2* gene expression at a cut-off value of ≥ 3.1 had a sensitivity of 50.0% and specificity of 49.09%. The AUC was 0.548, which was not statistically significant ($P = 0.479$) (Figure 5b).

Discussion

Acne vulgaris is an inflammatory skin disorder that affects the pilosebaceous unit. It may present as non-inflammatory lesions, inflammatory lesions, or a combination of both. The pathogenesis of acne is multifactorial and involves several factors: *C. acnes* activity, excess sebum production, and follicular hyperproliferation.[15]

Table 4. Relationship between mean *TIMP-2* gene expression level and clinical data of the studied cases (n = 83)

	n	*TIMP-2* gene		Test of sig.	*P*
		Mean ± SD	Median (min–max)		
Sex					
Male	**35**	3.86 ± 1.47	3.40 (1.35–6.50)	U = 753.500	0.423
Female	**48**	3.64 ± 1.56	3.05 (1.35–6.80)		
Onset					
Gradual	**56**	3.87 ± 1.48	3.20 (1.35–6.80)	U = 625.0	0.201
Acute	**27**	3.46 ± 1.57	3.0 (1.35–6.50)		
Course					
Stationary	**34**	3.70 ± 1.63	3.05 (1.35–6.80)	U = 795.0	0.724
Progressive	**49**	3.76 ± 1.44	3.20 (1.35–6.60)		
Aggravating factor					
No	**25**	3.46 ± 1.26	3.20 (1.35–6.50)	U = 643.0	0.413
Yes[#]	**58**	3.85 ± 1.61	3.15 (1.35–6.80)		
Spicy food	**21**	3.79 ± 1.57	3.20 (1.35–6.60)	U = 650.0	0.992
Sun	**35**	4.09 ± 1.81	3.10 (1.35–6.60)	U = 745.50	0.381
Stress	**52**	3.87 ± 1.65	3.05 (1.35–6.80)	U = 725.0	0.444
Menses	**36**	3.74 ± 1.68	3.15 (1.35–6.80)	U = 840.0	0.956
Family history					
No	**39**	3.87 ± 1.42	3.40 (1.35–6.50)	U = 721.0	0.209
Yes	**44**	3.62 ± 1.60	3.0 (1.35–6.80)		
Site[#]					
Face	**83**	3.74 ± 1.51	3.20 (1.35–6.80)	–	–
Back	**63**	3.92 ± 1.62	3.40 (1.35–6.60)	U = 463.50	0.075
Chest	**22**	4.18 ± 1.71	4.0 (1.35–6.50)	U = 462.50*	0.031*
NAST classification					
Non-inflammatory	**36**	2.86 ± 0.31	2.80 (2.30– 3.40)	U = 276.0*	< 0.001*
Inflammatory	**47**	4.41 ± 1.72	4.0 (1.35–6.80)		
Disease severity					
Mild	**27**	3.52 ± 1.26	3.0 (2.30–6.50)	K = 1.081	0.583
Moderate	**28**	3.90 ± 1.71	3.30 (1.35–6.60)		
Severe	**28**	3.78 ± 1.56	3.50 (1.35–6.80)		
GAGS grade					
Mild	**36**	2.86 ± 0.31	2.80 (2.30–3.40)	K = 48.799*	< 0.001*
Moderate	**24**	3.08 ± 1.0	3.10 (1.35–4.0)		
Severe	**23**	5.80 ± 1.08	6.30[ab] (3.40–6.80)		

SD: Standard deviation, U: Mann–Whitney test, TIMP-2: Tissue inhibitor of metalloproteinase-2, K: Kruskal–Wallis test, GAGS: Global Acne Grading System, Pairwise comparison bet. every 2 groups were done using post-hoc test (Dunn's test with Bonferroni adjustment for multiple comparisons)
n: Number, *P*: For comparison between diverse groups
*: $P \leq 0.05$ is the level of significance; X: Mean
[a]: Significance with mild, [b]: Significance with moderate

MMP-2 is a Zn+2-dependent endoproteinase that is involved in the disruption of sebaceous glands.[16] TIMP-2 is a unique inhibitor within the TIMP family that inhibits the proteolytic activity of MMP-2.[17]

The balance between TIMPs and MMPs controls the modification of the extracellular matrix and the imbalance between them may lead to the development of acne and other inflammatory disorder.[5]

Although TIMP-2 is classically recognized as an inhibitor of MMP-2, its increased expression may represent a compensatory response to enhanced extracellular matrix remodeling and inflammation associated with acne pathogenesis. Elevated TIMP-2 levels may reflect an attempt to counterbalance increased MMP activity and limit tissue damage during chronic inflammatory processes. Furthermore, TIMP-2 has been reported to exert biological functions beyond MMP inhibition, including regulation of cell proliferation, apoptosis, and tissue

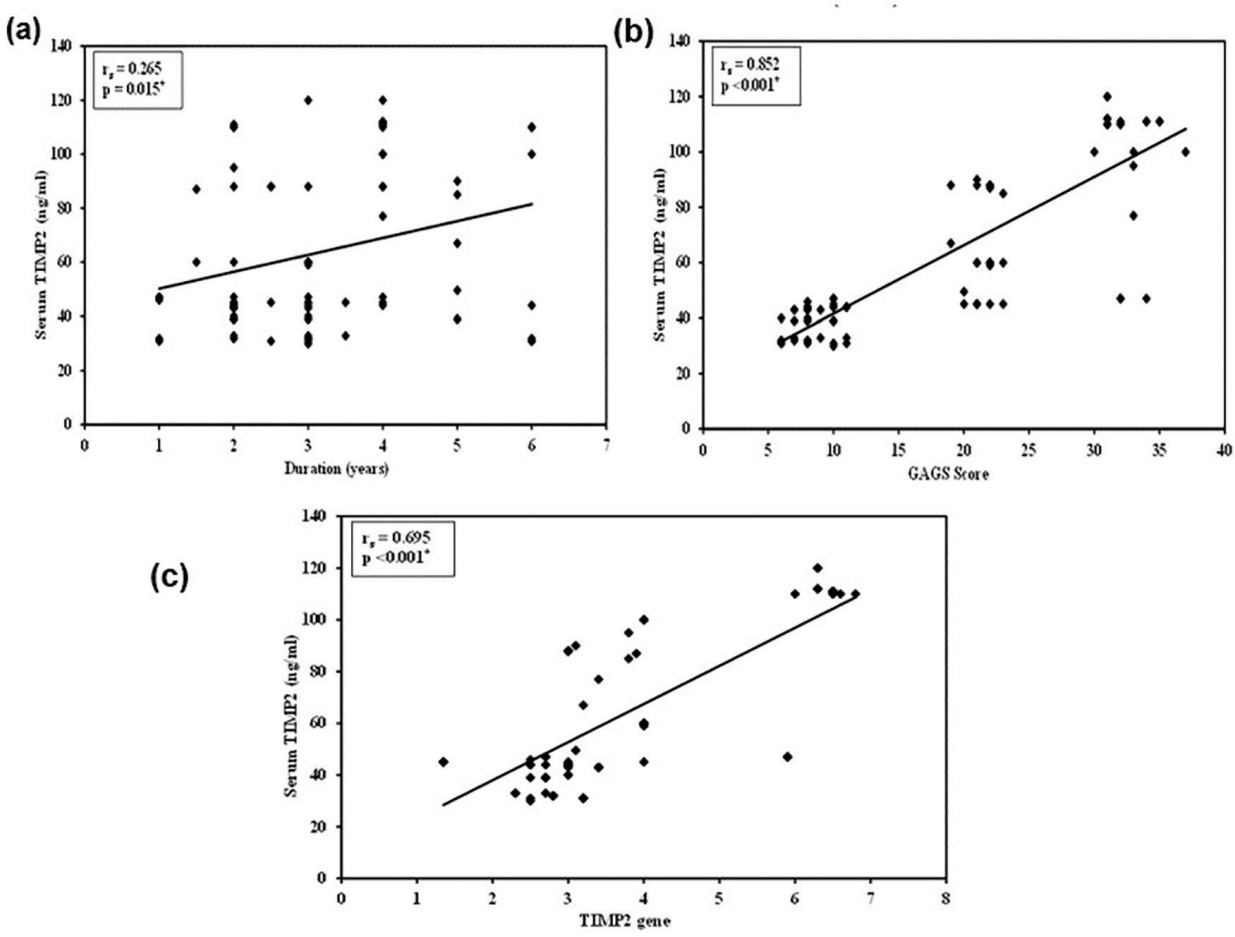

Figure 3. Correlation between serum TIMP-2 (ng/mL) and disease duration in years (r = 0.265, *P* = 0.015) (a), GAGS score (r = 0.852), (b) and serum *TIMP-2* level (r = 0.695), (*P* < 0.001 for both) and *TIMP-2* gene expression (c)
TIMP-2: Tissue inhibitor of metalloproteinase-2, GAGS: Global Acne Grading System

remodeling. Therefore, increased circulating TIMP-2 may serve as a marker of ongoing inflammatory and remodeling events rather than a direct pathogenic factor.[18]

The elevated serum TIMP-2 levels and gene expression observed in patients with acne vulgaris may represent a compensatory regulatory response to the inflammatory and extracellular matrix remodeling processes associated with the disease. As TIMP-2 is a major endogenous inhibitor of MMP-2 and plays an important role in maintaining extracellular matrix homeostasis, its upregulation may reflect an attempt to counterbalance increased proteolytic activity and tissue remodeling during inflammation. Similar regulatory functions of TIMP-2 have been described in several inflammatory and tissue-remodeling conditions.[19] Beyond its inhibitory activity against MMPs, TIMP-2 contributes to tissue homeostasis through MMP-independent mechanisms, including the regulation of cellular proliferation, angiogenesis, and tissue remodeling. Therefore, increased TIMP-2 expression may also reflect a broader protective response to ongoing inflammatory processes.[20] However, MMP-2 levels were not measured in the current study. Consequently, the proposed compensatory role of TIMP-2 cannot be directly confirmed, and our findings should be interpreted as demonstrating an association between increased TIMP-2 levels and acne severity rather than establishing a causal mechanism. Future studies that evaluate both TIMP-2 and MMP-2 are warranted to clarify their interaction in the pathogenesis of acne vulgaris.

Papakonstantinou et al.[21] investigated the expression of MMPs and their inhibitors in the facial sebum of patients with acne, both before and after isotretinoin treatment, and documented the presence of TIMP-2 in the sebum of acne patients, which was not influenced by isotretinoin treatment. Other studies investigated TIMP-2 tissue expression in acne patients, including Saint-Jean et al.[22] which found that TIMP-2 expression was significantly stronger in papules of patients prone to acne scars compared to patients not prone to scars.

The expression of TIMP-2 was studied only in sebum and tissue cultures, but not in the blood of acne vulgaris

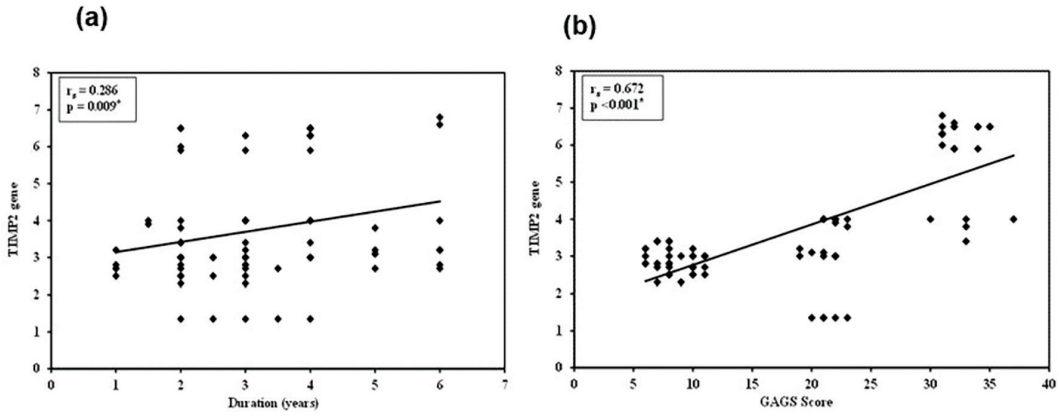

Figure 4. Correlation between *TIMP-2* gene expression and disease duration in years (a) (r = 0.286), and GAGS score (b) (r = 0.672)
TIMP-2: Tissue inhibitor of metalloproteinase-2, GAGS: Global Acne Grading System

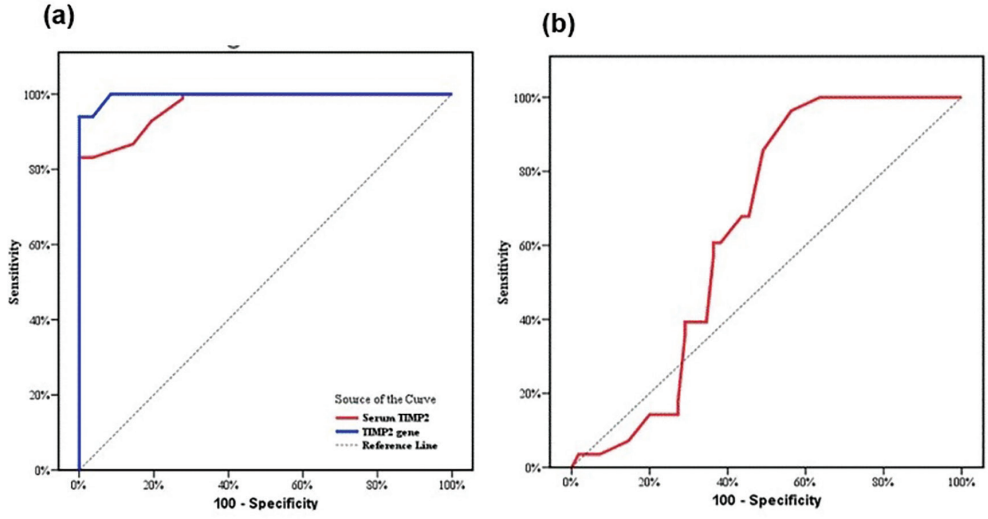

Figure 5. (a) ROC curve for serum TIMP-2 (ng/mL) and *TIMP-2* gene expression to discriminate cases from controls. (b) ROC curve for serum TIMP-2 and *TIMP-2* gene expression to discriminate disease severity
ROC: Receiver operating characteristic, TIMP-2: Tissue inhibitor of metalloproteinase-2

patients,[21,22] whereas the current study aimed to assess serum TIMP-2 levels in cases of acne vulgaris to better understand its role in acne vulgaris etiopathogenesis.

This study found that mean serum TIMP-2 levels were significantly higher in acne vulgaris patients than in control subjects. Indeed, a significant relationship between the mean serum TIMP-2 level and aggravating factors, such as sun exposure and pregnancy, was observed.

This may be explained by the rising TIMP-2 level, which decreases MMP-2. Gao et al.[16] stated that there may be an increase in proinflammatory cytokine production leading to systemic inflammation if MMP-2 activity declines below baseline. Additionally, they reported that lipid dysregulation, including decreased liver X receptor-α levels and increased hepatic triglyceride production, was caused by MMP-2 deficiency, and that similar dysregulation was also reported in acne.

The concentration of TIMP-2 is higher in acne lesions on the back and chest. Dagnelie et al.[23] reported that cases with acne on the back exhibit a loss of *C. acnes* phylotypes, particularly phylotype IA1, thereby activating innate immunity. Saint-Jean et al.[22] documented a distinct innate immunity profile in the normal skin of back acne patients with and without acne scars. In patients who developed scars, considerable overexpression of TLR-4, interleukin (IL)-2, IL-10, TIMP-2, and the transcription factor JUN, alongside decreased MMP-9 protein levels, was observed.

The results of this investigation showed significant positive correlations between mean serum TIMP-2 levels and both disease duration (years) and NAST classification; TIMP-2

serum levels were significantly higher among patients with prolonged disease duration and inflammatory lesions. This finding was consistent with Gao et al.,[16] who confirmed that inhibited MMP-2 activity may lead to overexpression of IL-1α and β, tumor necrosis factor-α, and triglycerides, thus promoting inflammation and lipid dysregulation in acne.

The current study supported the use of TIMP-2 as a marker for the diagnosis of acne vulgaris, with a cut-off value of ≥ 31, yielding a sensitivity of 92.77%, specificity of 80.72%, and an AUC of 0.969, with a statistically significant result ($P < 0.001$). It can also be used to detect the severity of acne vulgaris, as serum TIMP-2 shows significant diagnostic performance in discriminating between severe and mild/moderate disease at a cut-off of ≥ 47, with a sensitivity of 60.71% and specificity of 61.82%. AUC was 0.645 with a statistically significant result ($P = 0.031$)

This suggests that acne patients with high TIMP-2 levels are more likely to develop severe, extensive disease and scarring. Chuah and Goh[24] suggested that predicting the susceptibility to severe acne is very important, as early treatment is easier and prevents the serious side effects of post-acne scarring.

Many studies have investigated the *TIMP*-2 gene polymorphism in acne vulgaris. Yaykasli et al.[10] documented twofold higher expression of the *TIMP-2-418CC* genotype in cases with acne vulgaris.

Furthermore, Gao et al.[16] conducted a study of the Chinese population and found no significant correlation between the TIMP-2-41/C polymorphism and acne vulgaris. Gupta et al.[25] found no evidence of increased risk of acne or atrophic post-acne scars associated with the TIMP-2 nucleotide polymorphism (rs8179090).

Moreover, *TIMP-2* mRNA expression is associated with many skin disorders, including psoriasis,[26] systemic lupus erythematosus,[27] non-melanoma skin cancer,[28] and oral lichen planus.[29] However, the association between *TIMP-2* mRNA expression and the occurrence of acne vulgaris has not been studied to date.

The current study showed a significant difference between patients with acne vulgaris and control subjects, with *TIMP-2* gene expression levels higher in patients than in control subjects.

The results of the present study showed significant associations between mean *TIMP-2* gene expression levels and acne lesions on the chest, NAST classification, and disease duration (years), with higher levels observed in patients with chest lesions, inflammatory acne, and longer disease duration.

The current study supported the use of *TIMP-2* gene expression as a marker for the diagnosis of acne vulgaris, with a cut-off value of ≥ 1.5, demonstrating 93.98% sensitivity and 100.0% specificity. The AUC was 0.996, which was statistically significant.

A significant positive correlation was observed between the mean serum TIMP-2 level and *TIMP-2* gene expression. The *TIMP-2* gene encodes the TIMP-2 protein; therefore, increased expression of the *TIMP-2* gene results in higher levels of its protein product.

In the current study, the mean serum TIMP-2 level was significantly associated with disease severity, GAGS grade, and score. Indeed, a significant positive correlation between *TIMP-2* gene expression and GAGS grade and score was documented.

Both *TIMP-2 gene* expression and serum levels were higher in patients with severe acne, defined by a high GAGS score using lesion type rather than lesion count. This finding can be explained by the likelihood that acne lesions may leave permanent scars, which increases with the depth of the inflammatory process.[30] In line with this finding, Wen et al.[31] revealed that the risk of acne scarring was associated with *TIMP-2* (rs4789932).

Study Limitations

The current study has some limitations. First, MMP-2 levels were not assessed; therefore, the functional relationship between TIMP-2 and MMP-2 could not be directly investigated. Second, the case-control design precludes establishing causal relationships between elevated TIMP-2 levels and acne development or progression. Future longitudinal studies incorporating simultaneous measurement of TIMP-2 and MMP-2 are recommended to clarify the underlying mechanisms.

Conclusion

The present study demonstrated that both serum TIMP-2 levels and *TIMP-2* gene expression are significantly elevated in patients with acne vulgaris compared with healthy individuals. Furthermore, the positive correlations of serum TIMP-2 levels and its gene expression with acne severity scores (GAGS and NAST classification) suggest a probable role for TIMP-2 in the pathogenesis and progression of acne vulgaris. These findings indicate that TIMP-2 may serve as a useful biomarker of disease activity and severity, highlighting its potential utility in the evaluation and management of acne vulgaris. Further studies are warranted to elucidate the underlying mechanisms and explore its potential as a therapeutic target.

Ethics

Ethics Committee Approval: Prior to the initiation of the study, ethical approval was obtained from the Research Ethics Committee, Institutional Review Board, Faculty of Medicine, Menoufia University (approval no: DERMA 27; approval date: 27 October 2022). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Informed Consent: Each participant provided written informed consent before the start of the study, which was approved by the local research ethics committee. One of the patients' parents provided consent for the patients under the age of eighteen.

Authorship Contributions

Design: W.S., M.B., Data Collection or Processing: M.A.E-K., Analysis or Interpretation: S.E., S.S.E., Literature Search: M.A.E-K., Writing: S.E., S.S.E.

Footnotes

Conflict of Interest: The authors declared that they have no conflict of interest.

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Yang S, Fang F, Yu X, Yang C, Zhang X, Wang L, Zhu L, Shao K, Zhu T. Knockdown of H19 inhibits the pathogenesis of acne vulgaris by targeting the miR-196a/TLR2/NF-κB axis. Inflammation. 2020;43(5):1936-1947.
2. Agrawal DA, Khunger N. A morphological study of acne scarring and its relationship between severity and treatment of active acne. J Cutan Aesthet Surg. 2020;13(3):210-216.
3. Lynn DD, Umari T, Dunnick CA, Dellavalle RP. The epidemiology of acne vulgaris in late adolescence. Adolesc Health Med Ther. 2016;7:13-25.
4. Fouda I, Obaid ZM, Hegazy SF, Samir Abd Al-Samie H, Nofal A. Calprotectin in acne vulgaris: a possible contributory role. J Cosmet Dermatol. 2021;20:621-625.
5. Zhang H, Zhang Z. Genetic variants associated with acne vulgaris. Int J Gen Med. 2023;16:3843-3856.
6. Gelzo M, Cacciapuoti S, Pinchera B, De Rosa A, Cernera G, Scialò F, Comegna M, Mormile M, Fabbrocini G, Parrella R, Corso G, Gentile I, Castaldo G. Matrix metalloproteinases (MMP) 3 and 9 as biomarkers of severity in COVID-19 patients. Sci Rep. 2022;12(1):1212.
7. Wang K, Wang G, Huang S, Luo A, Jing X, Li G, Zhou Y, Zhao X. Association between TIMP-2 gene polymorphism and breast cancer in Han Chinese women. BMC Cancer. 2019;19(1):446.
8. Wang DD, Xu WX, Chen WQ, Li L, Yang SJ, Zhang J, Tang JH. Identification of TIMP2 as a prognostic biomarker and its correlation with tumor immune microenvironment: a comprehensive pan-cancer analysis. J Oncol. 2022;2022:9133636.
9. Guo T, Hao H, Zhou L, Zhou F, Yu D. Association of SNPs in the TIMP-2 gene and large artery atherosclerotic stroke in southern Chinese Han population. Oncotarget. 2017;9(4):4698-4706.
10. Yaykasli KO, Turan H, Kaya E, Hatipoglu OF. Polymorphisms in the promoters of MMP-2 and TIMP-2 genes in patients with acne vulgaris. Int J Clin Exp Med. 2013;6(10):967-972.
11. Nast A, Dréno B, Bettoli V, Bukvic Mokos Z, Degitz K, Dressler C, Finlay AY, Haedersdal M, Lambert J, Layton A, Lomholt HB, López-Estebaranz JL, Ochsendorf F, Oprica C, Rosumeck S, Simonart T, Werner RN, Gollnick H. European evidence-based (S3) guideline for the treatment of acne - update 2016 - short version. J Eur Acad Dermatol Venereol. 2016;30(8):1261-1268.
12. Tan JKL. Current measures for the evaluation of acne severity. Expert Review of Dermatology. 2008;3(5):595-603.
13. Doshi A, Zaheer A, Stiller MJ. A comparison of current acne grading systems and proposal of a novel system. Int J Dermatol. 1997;36(6):416-418.
14. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001;25(4):402-408.
15. Farag AGA, Maraee AH, Rifaat Al-Sharaky D, Elshaib ME, Kohla MSM, Shehata WA. Tissue expression of IL-17A and FOXP3 in acne vulgaris patients. J Cosmet Dermatol. 2021;20(1):330-337.
16. Gao R, Yu H, Zhao Q, Wang S, Bai B. Role of MMP-2(-1306 C/T) and TIMP-2(-418G/C) polymorphism in Chinese han patients with acne vulgaris. Biomed Res Int. 2019;2019:2364581.
17. Jian F, Yanhong J, Limeng W, Guoping N, Yiqing T, Hao L, Zhaoji P. TIMP2 is associated with prognosis and immune infiltrates of gastric and colon cancer. Int Immunopharmacol. 2022;110:109008.
18. Brew K, Dinakarpandian D, Nagase H. Tissue inhibitors of metalloproteinases: evolution, structure and function. Biochim Biophys Acta. 2000;1477(1-2):267-283.
19. Brew K, Nagase H. The tissue inhibitors of metalloproteinases (TIMPs): an ancient family with structural and functional diversity. Biochim Biophys Acta. 2010;1803(1):55-71.
20. Stetler-Stevenson WG. The tumor microenvironment: regulation by MMP-independent effects of tissue inhibitor of metalloproteinases-2. Cancer Metastasis Rev. 2008;27(1):57-66.
21. Papakonstantinou E, Aletras AJ, Glass E, Tsogas P, Dionyssopoulos A, Adjaye J, Fimmel S, Gouvousis P, Herwig R, Lehrach H, Zouboulis CC, Karakiulakis G. Matrix metalloproteinases of epithelial origin in facial sebum of patients with acne and their regulation by isotretinoin. J Invest Dermatol. 2005;125(4):673-684.
22. Saint-Jean M, Khammari A, Jasson F, Nguyen JM, Dréno B. Different cutaneous innate immunity profiles in acne patients with and without atrophic scars. Eur J Dermatol. 2016;26(1):68-74.
23. Dagnelie MA, Corvec S, Saint-Jean M, Nguyen JM, Khammari A, Dréno B. Cutibacterium acnes phylotypes diversity loss: a trigger for skin inflammatory process. J Eur Acad Dermatol Venereol. 2019;33(12):2340-2348.
24. Chuah SY, Goh CL. The impact of post-acne scars on the quality of life among young adults in Singapore. J Cutan Aesthet Surg. 2015;8(3):153-158.
25. Gupta N, Bishnoi A, Mathew D, Arora A, Handa S, Kaur J, De D, Pal A. Hypertrophic post-acne scarring is associated with a single nucleotide polymorphism (rs243865) in the matrix metalloproteinase-2 gene. J Dtsch Dermatol Ges. 2020;18(12):1426-1435.
26. Wagner MFMG, Theodoro TR, Filho CASM, Oyafuso LKM, Pinhal MAS. Extracellular matrix alterations in the skin of patients affected by psoriasis. BMC Mol Cell Biol. 2021;22(1):55.
27. Vira HJ, Pradhan VD, Umare VD, Chaudhary AK, Rajadhyksha AG, Nadkar MY, Ghosh K, Nadkarni AH. Role of MMP-2 and its inhibitor TIMP-2 as biomarkers for susceptibility to systemic lupus erythematosus. Biomark Med. 2020;14(12):1109-1119.
28. O'Grady A, Dunne C, O'Kelly P, Murphy GM, Leader M, Kay E. Differential expression of matrix metalloproteinase (MMP)-2, MMP-9 and tissue inhibitor of metalloproteinase (TIMP)-1 and TIMP-2 in non-melanoma skin cancer: implications for tumour progression. Histopathology. 2007;51(6):793-804.

29. Mazzarella N, Femiano F, Gombos F, De Rosa A, Giuliano M. Matrix metalloproteinase gene expression in oral lichen planus: erosive vs. reticular forms. J Eur Acad Dermatol Venereol. 2006;20(8):953-957.
30. Eichenfield DZ, Sprague J, Eichenfield LF. Management of acne vulgaris: a review. JAMA. 2021;326(20):2055-2067.
31. Wen X, Du H, Hao X, Zhang H, Guo Y. TIMP2 genetic variation rs4789932 may associate with an increased risk of developing acne scarring based on a case-control study of Chinese Han population. J Cosmet Dermatol. 2022;21(10):4740-4747.