INTRODUCTION

Acne vulgaris is a multifactorial inflammatory skin condition resulting from the integration between multiple factors including androgen-induced increase in sebum secretion, altered keratinization, inflammation, pilosebaceous unit colonization by Cutibacterium acnes (C. acnes), delayed-type immune reaction, diet, and genetic factors.\(^1\)

The proteolytic enzymes, matrix metalloproteinases (MMPs) and intrinsic tissue inhibitors of metalloproteinases (TIMPs), regulate extracellular matrix (ECM) remodeling and inflammatory signaling.
They also have a role in controlling multiple cellular events including cell proliferation, differentiation, and apoptosis.\textsuperscript{2,3}

Different MMPs family members may be implicated in acne vulgaris development. In acne lesions, production of several MMPs (eg, MMP-1, 2, 3, 9, and 13) is induced by the effect of \textit{C. acnes} on keratinocytes.\textsuperscript{4}

Published data about the role of MMP-1 and TIMP-1 genes polymorphisms in acne vulgaris development or severity are scarce.

The aim of the current study was to evaluate the association between MMP-1 (519 A/G) and TIMP-1 (372 T/C) genes polymorphisms and the risk to develop acne vulgaris among a sample of Egyptian acne patients.

2 | PATIENTS/METHODS

2.1 | Ethical Considerations

This case-control study was approved by the local ethical committee of research (approval code: MS 1.1.2017). Informed consents to participate in the study and to publish the collected data were obtained from all participants prior to joining the study.

2.2 | Subjects

The study included 220 subjects: 100 patients suffering from acne vulgaris (AV) (Group A). Another group (group B) of 120 apparently healthy, acne-free individuals (free from active acne lesions and with negative history of acne) were included as the patient group. They were of matched age, sex, and BMI as a control group. Patients were recruited from the outpatient clinic of Dermatology Department.

Subjects with acniform eruption, acne conglobata, fulminating acne, and polycystic ovary syndrome were excluded from the study. Those with inflammatory or autoimmune cutaneous or systemic diseases, malignancy, and chronic systemic diseases were also excluded. Using topical anti-acne treatment within 2 weeks before sample collection or systemic anti-acne treatment within 2 months before sample collection was also considered among exclusion criteria.

2.3 | Methods

2.3.1 | Clinical Evaluation

All patients were subjected to the following: full history taking and general clinical examination. PCOS was diagnosed by the Rotterdam criteria which require the presence of two criteria of the following: oligo/anovulation, hyperandrogenism or polycystic ovaries on ultrasound.\textsuperscript{5} Complete cutaneous examination was performed to evaluate the distribution and severity of AV according to the Global Acne Grading System (GAGS)\textsuperscript{6} and to detect the presence of postacne scars.

2.3.2 | DNA extraction and genotyping

Blood samples from all participants were collected in a 2 ml EDTA tubes. Genomic DNA was obtained from peripheral blood leukocytes by a standard method and stored at 4°C prior to genomic DNA extraction using a quick-DNATM miniprep kit (catalog Nos D3007, D3024, and D2025) according to the manufacturer's instructions. The collected samples were kept at −20°C. Samples analysis was performed using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique.

Genotypes of the A/G polymorphism in the MMP-1 promoter were determined by PCR-based KpnI restriction fragment length polymorphism, and 200 ng DNA was used as the template in PCR using the primers forward 5'-CAT GGT GCT ATC GCA ATA GGG T-3' and reverse 5'-TGC TAC AGT TTT TTC CTC CAC ACA C-3', polymorphism of the MMP-1 519 A/G (rs 1144393). The amplification conditions were 1 min at 95°C, followed by 30 cycles of 30 s at 94°C, 30 s at 55°C, and 30 s at 72°C, by PikoReal 24 Thermo scientific and Taq TM Red mix (cat #Bio-25043 Bioline, UK).

The amplified DNA was restricted with endonuclease KpnI (New England Biolabs, Boston, USA,) for 2 h at 37°C.

PCR fragments were analyzed comparing with 100 bp DNA ladder on an ethidium bromide stained 2% agarose gel electrophoresis on Biometra gel electrophoresis unit and visualized under ultraviolet light transillumination.

KpnI was used to digest the product (200 bp). The A allele corresponds to the 176 bp and 24 bp fragments. The G allele corresponds to the 200 bp.

About 200 ng DNA was used as the template in PCR using the primers forward 5'-GCACATCAGCTGAGTCT-3' and reverse 5'-GAAACAAGCCCAGATTAG-3', flanking the 372 T/C polymorphism (rs4898) of the TIMP-1 gene, and the amplification conditions were one minute at 95 degree Celsius, followed by 30 cycles of 30 s at 94 degree Celsius, 30 s at 55 degree Celsius, and 30 s at 72 degree Celsius by PikoReal 24 Thermo scientific, Taq TM Red mix (cat #Bio-25043 Bioline, UK) (C means a mutation occurred in the primer to form a BssSI restriction site (CAGCAG) in the C allele, while the T allele stay uncut). Endonuclease BssSI was used to restrict the amplified DNA (New England Biolabs, Boston, USA, lot:0061703) for two hours at 37 degree Celsius. To separate the resultant fragments of DNA, gel electrophoresis in 2% agarose gel and ultraviolet light was performed. In the absence of a BssSI site, a fragment of 175 base pairs was detected (T allele), whereas fragments of 153 and 22 base pairs corresponded to the C allele.

2.3.3 | Statistical analysis

The collected data were tabulated and statistically analyzed using Statistical package for Social Science (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.). Parametric numerical data were presented in the form of mean ± standard deviation (SD), while non-numerical data were...
presented as frequency and percentage. \( p \)-value was considered significant if \( <0.05 \).

- Student’s \( t \)-test was used to assess the statistical significance of the difference between two study group means.
- For the comparison of the three groups’ means, one-way analysis of variance (ANOVA) was used followed by Tukey test.
- Chi-Square test was used to examine the relationship between two qualitative variables
- Fisher’s exact test was used to examine the relationship between two qualitative variables when the expected count is less than 5 in more than 20% of cells.
- Deviations from Hardy-Weinberg equilibrium: expectations were determined using the chi-squared test.
- Odds ratio and 95% confidence interval were calculated

### RESULTS

#### 3.1 Sociodemographic and clinical data

There was insignificant difference between patients and control subjects regarding gender; 73 (73%) versus 78 (65%) females, \( p = 0.20 \), age \( (19.25 \pm 3.9 \) versus 20.2 \( \pm 3.7 \) years old, \( t = 1.8, p = 0.06 \), and BMI \( (22.45 \pm 2.16 \) versus 22.62 \( \pm 2.8 \) kg/m\(^2\), \( t = 0.49, p = 0.62 \).

Sixty-seven cases (67%) reported a history of acne vulgaris in their families. The mean age at the disease onset was 15.23 \( \pm 2.74 \) years, and the mean disease duration was 4.04 \( \pm 2.33 \) years. All patients (100%) suffered from acne on the face, while back was affected in 50 patients (50%) only. Acne vulgaris was severe in 50 patients (50%), moderate in 30 patients (30%), and 20 patients (20%) presented with mild acne. Postacne scar formation was detected in 63 cases (63%).

#### 3.2 Genotypes and Alleles Distribution

Both patients and control subjects groups were in Hardy-Weinberg equilibrium regarding both studied SNPs. The \( p \)-value for Hardy-Weinberg equilibrium for MMP-1 (519 A/G) and TIMP-1 (372 C/T) in acne group was 0.06 and 0.07 respectively, while in the control group, it was 0.09 and 0.84, respectively.

Results of the current study showed that MMP-1 (519 A/G) gene polymorphism increases the risk of acne vulgaris ~fourfolds (Figure 1). In females, TIMP-1 (372 C/T) TT genotype and T allele were significantly predominant in cases when compared to the control group \( (p = 0.004, 0.001 \) respectively) with higher risk to develop acne \( (OR = 16 \) and 3.26, respectively) (Figure 2). On the other hand, there was insignificant difference between the frequency of alleles in patients and control male subjects (Figure 3).

Among the studied variables, TIMP-1 (372C/T) TT genotype has been shown to be significantly detected in the studied female patients associated with positive family history of the disease, the risk of back affection, the severe acne grades, and the liability to post-acne scar formation (Table 1). There were insignificant differences in TIMP-1 (372C/T) alleles distribution according to demography, history findings, and the clinical findings in the studied male acne patients.

### DISCUSSION

The present work studied the MMP-1 (519A/G) and TIMP-1 (372 T/C) genes polymorphisms in a sample of Egyptian acne vulgaris patients. MMP-1 gene is located on the long arm of chromosome 11 (11q22.3).\(^7\) The ancestral allele at this position is the A allele. It was demonstrated in vitro that the presence of G allele
The MMPs gene polymorphisms have been shown to be related to many skin disorders including squamous cell carcinoma, oral lichen planus, cutaneous melanoma, and recessive dystrophic epidermolysis bullosa. However, the association between these genes polymorphisms and acne vulgaris development or its clinical criteria has not been studied yet.

In the present work, a link between MMP-1 (519 A/G) gene polymorphism and acne vulgaris was detected. MMP-1 (519 A/G) genotypes and alleles distribution were significantly different between acne patients and control subjects groups. AG and GG genotypes and G allele seem to increase the risk of developing acne vulgaris.

Polycystic ovary syndrome is associated with different skin manifestations related to hormonal changes including acne vulgaris. Walch et al. studied the same SNP in polycystic ovary syndrome patients. They detected an association between the risk of PCOS and GG genotype of the same SNP of MMP-1 gene. Further larger studies investigating the genotypes distribution of this SNP in female acne patients with and without PCOS may be needed.

Nho et al. found that the frequency of MMP-1 (519 A/G) G allele is significantly higher in subjects with BMI < 25.0 Kg/m² suggesting a protective role of MMP-1 (519 A/G) G allele against the increase in BMI in Korean population. However, the relation between the G allele and BMI in the current patients’ sample cannot be evaluated accurately due to the normal overall mean of BMI in the study sample.
Checa et al. reported an association between the polymorphism of the MMP-1 gene promoter with the development of idiopathic pulmonary fibrosis. In the present study, there was no relation between MMP-1 (519 A/G) gene polymorphism and the postacne scar formation. This might be because the process of scar formation depends on the imbalance between the MMP and its tissue inhibitor than rather than its own activity alone.

Tissue inhibitor of metalloproteinase-1 (TIMP-1) gene is located at chromosome X (Xp11.23–11.4), which means that men have only either the C or T alleles, while women have CC, CT, or TT genotypes. The ancestral allele is the C allele. Patients with T allele show higher serum levels of TIMP-1. The association between this TIMP-1 polymorphism and other diseases was reported in previous studies, for example, systemic sclerosis, sepsis, chronic obstructive pulmonary disease, and primary open-angle glaucoma. However, there is scarcity in the studies investigating the association between this polymorphism and dermatological disorders.

In the present study, TIMP-1 (372 C/T) TT genotype and T allele showed significantly higher frequency in female cases when compared to the females in the control group. While in male participants, there was insignificant difference in the alleles’ distribution between patients and control subjects.

The TT genotype of TIMP-1 (372 C/T) was predominant in female patients with positive family history of acne vulgaris. It was suggested that the positive family history of acne vulgaris is associated with increased risk of earlier onset and more noninflammatory lesions. The current results suggest that the TT genotype of this SNP has a role in the familial cases of acne vulgaris. This is further evidenced by the fact that male patients carry only either C or T allele.

Among female patients carrying the TIMP-1 (372 C/T) CT and TT genotypes (polymorphic genotypes), the back was affected in 77.5%, and 100% of them showed severe form of acne and postacne scar formation. This suggests that acne patients carrying TIMP-1 (372 C/T) gene polymorphism are more vulnerable to develop severe extensive acne vulgaris and are more liable to form postacne scars. It would be of great importance if we can predict the susceptibility for severe acne development and postacne scars formation. Treating the patients susceptible to severe acne and postacne scar formation properly from the start could be much easier than treating these difficult situations and their deleterious effects on QoL. We can propose that acne patients who have TIMP-1 (372 C/T) polymorphic genotypes may need aggressive treatment from the start in order to limit the disease distribution, alleviate disease severity, and prevent postacne scar formation.

In the present study, there was insignificant difference between male patients and control subjects regarding TIMP-1 (372 C/T) polymorphism. This means that the T allele alone cannot induce acne development. In female patients, CT genotype carriers have no increased risk of acne vulgaris which supports the importance of TT genotype.

Away from the effect on extracellular matrix, MMP and TIMP have different immune-regulatory effects. In inflammatory conditions, MMP may contribute to the ongoing inflammatory process via regulating different cytokines (eg, TNF-α and IL-β) production and activity. TIMP-1 also can bind to certain receptors to perform cytokine functions independent from MMP. There might be a synergism between TIMP-1 and TNF/IFNγ, in certain conditions. These previous studies together with the results of the present work suggest that MMP-1 and TIMP-1 gene polymorphisms may be involved in acne vulgaris development and in modification of its clinical aspects.

Although aggressive therapy or choosing therapeutic lines of high-grade acne in lower ones is currently accepted and even suggested by the experts of isotretinoin, worries about its side effects, misconception, and lack of proper awareness may make patients refuse using it and even their parents may have a prejudice about prescribing it to their children. Results of the current study suggest that if dermatologists could detect patients who will most probably form postacne scars, they would be more able to convince them to use this aggressive therapy as a protective measure or even as an early treatment line as suggested for pigmented post-varicella scars. However, clinically, it is difficult to predict which acne vulgaris case will develop postacne scarring. It was thought that scars mainly follow nodulocystic acne lesions, but it has been shown that even noninflammatory acne lesions and mild degrees of acne can

<table>
<thead>
<tr>
<th>Variables</th>
<th>MMP (519 A/G)</th>
<th>TIMP1 (372C/T) in female patients</th>
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<tbody>
<tr>
<td></td>
<td>A/A N = 40</td>
<td>(A/G)+(G/G) N = 60</td>
</tr>
<tr>
<td>Family history, N (%)</td>
<td>25 (37.3)</td>
<td>42 (62.6)</td>
</tr>
<tr>
<td>Back affection, N (%)</td>
<td>18 (36)</td>
<td>32 (64)</td>
</tr>
<tr>
<td>Scar formation, N (%)</td>
<td>22 (34.9)</td>
<td>41 (65.07)</td>
</tr>
</tbody>
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*: significant.
cause disfiguring postacne scarring.\textsuperscript{33} That’s why it would be of great benefit to find out more reliable laboratory markers\textsuperscript{34} or genetic factors which would help in expecting patients susceptible to postacne scarring formation more accurately. The small sample size and not correlating different genotypes with the tissue expression of MMP-1 and TIMP-1 are the main study limitations.

In conclusion, MMP-1 (519 A/G) and TIMP-1 (372 T/C) gene polymorphisms may be related to acne vulgaris development. Further studies are warranted to prove or refute the association between TIMP-1 (372 C/T) gene polymorphism and acne vulgaris development and risk of subsequent scarring in larger patients samples of different ethnic group. Studies correlating MMP-1 and TIMP-1 genes polymorphism with tissue expression of their proteins are also recommended.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Shimaa Moustafa Ali Mohammed involved in cases recruitment and review of literature. Hanan Hassan Sabry, MD involved in the study idea and results analysis and discussion. Seham Gouda Ameen, MD involved in the laboratory work, results discussion. Rehab Mohammed Salem, MD involved in the study design, results discussion, statistical analysis, and submitting the work.

ETHICAL APPROVAL

This case-control study was approved by the local ethical committee. Informed consent was obtained from each individual before sample collection.

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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