INTRODUCTION

Acne Vulgaris (AV) is a chronic inflammatory disease of the pilosebaceous unit. It is classified into two groups: noninflammatory (open and closed comedones) and inflammatory acne (papules, pustules, nodules, and cysts), with papules considered an intermediary step between the two. The pathogenesis is multifactorial, with an important role of inflammatory mediators and pro-inflammatory cytokines in its pathogenesis. These inflammatory cytokines produced by keratinocytes, macrophages, and neutrophils induce hyperkeratinization, comedones, and inflammatory lesions because of CD4 T-cell activation and migration. During puberty alteration of the sebaceous lipid profile, aggravated by stress and irritation, induce inflammation and AV development. Another player in the pathogenesis is the pro-inflammatory activity of the cutaneous microbiome. Dysbiosis leads to a disturbed barrier and disequilibrium of skin microbiome. Kruppel-like factors (KLF)s share the homology of proteins to DNA-binding domains of Drosophila fly; Drosophila embryos deficient in kruppel die due to abnormal thoracic and abdominal segmentation and appear crippled (kruppel means cripple).
family of KLF has 18 members, with a wide range of expression profile among several tissues. Each of which contains 3 zinc fingers by that bind to the DNA-binding transcription factors, which play role in proliferation, differentiation, development, growth, and inflammation. Kruppel-like factors are widely expressed in various tissues, genes only can encode transcription activators and repressors proteins.

Kruppel-like factor 2 gene, located at chromosome 19 p13.1, plays an important role in regulating adipogenesis, inflammatory, and malignant conditions. The KLF2 is expressed in positive CD4 and CD8 cells and remains highly expressed in both naïve and memory T cells. It has been shown that during T-cell stimulation, both KLF2 mRNA and protein are downregulated.

An important process that triggers AV is that Propionibacterium acnes (P. acnes) activates the innate immunity via expression of protease-activated receptors (PARs), TNF-α, and toll-like receptors (TLRs), and the production of interferon (INF), interleukins (IL-8, IL12, IL-1), and matrix metalloproteinases (MMPs) resulting in subsequent hyperkeratinization of the pilosebaceous unit. Downregulated KLF2 leads to rapid secretion of cytokines such as IL-1β, IL-8, TNF-α, and monocyte chemotactic protein-1 (MCP-1), IL-6 and MMP, which suggests that KLF2 might be linked to pathogenesis of AV.

THE AIM OF THE STUDY

The aim was to assess the role of KLF2 in pathogenesis of inflammatory acne.

SUBJECTS AND METHODS

This case-control study included 100 clinically diagnosed AV patients with different severities and 50 age- and sex-matched healthy controls. The inflammatory acne patients were 19 males and 31 females (age: 22.50 ± 3.62 years). Noninflammatory acne patients were 21 males and 29 females (age: 26.08 ± 4.91). Healthy controls were 26 males and 24 females (age: 25.84 ± 4.89 years). Patients were selected from outpatient clinic of Dermatology Department. Written informed consent was taken before the start of the study, which was approved by local Ethics Committee for Human Research in Faculty of Medicine in accordance with Helsinki declaration of the human rights 1975. Patients were excluded if suffering from other systemic inflammatory diseases, autoimmune diseases, infectious diseases or reported previous treatment with oral retinoids or any medications affects the AV severity. Each patient was subjected to complete history taking, general examination, and clinical assessment of AV. Severity of AV was assessed by using the Global Acne Severity Scale.

Sampling: Two ml venous blood were obtained from each subject, put into sterile tube with EDTA, mixed well, and aliquoted into two eppendorf tubes. Tubes were kept at −80°C till the following steps.

3.1 | Relative quantitation of KLF2 mRNA level

A. Total RNA extraction: Total RNA extraction was performed with 100 µL EDTA whole blood via Direct-zol RNA MiniPrep (Zymo Research). Digestion with DNase I was performed. Ultraviolet spectrophotometric quantification of RNA by Nanodrop 2000 Spectrophotometer (Thermo Fisher Scientific). Pure RNA preparations have optical density (OD) ratio at 260/280 nm of 1.9-2.3.

B. Quantitative real-time polymerase chain reaction (qRT-PCR): It was performed on two-steps: The first step is conversion of RNA into complementary DNA (cDNA) in a Veriti™ Thermal Cycler (Applied Biosystems), using Sensifast™ cDNA Synthesis Kit (Bioline Reagents Ltd). The PCR mix for reverse transcription reaction contained 5 µL RNA extract, 4 µL Transamp Buffer (5x), 1 µL reverse transcriptase and 10 µL nuclease-free water. The thermal cycler conditions were 25°C for 10 minutes, 42°C for 15 minutes, and 85°C for 5 minutes. The second step was quantitation of KLF2 mRNA in a Stepone real-time PCR system (Applied Biosystem). Singleplex reactions were done. This step was performed using TransStart Green qPCR SuperMix (TransGen Biotech Co., Ltd). Human GAPDH was the endogenous housekeeping gene. Melting curve analysis was done in each run to confirm specificity of real-time PCR assay. KLF2 primers were as follows: Forward primer (FP): 5′-ACAGACTGCTATTTATGGACCTTAG-3′ & Reverse primer (RP): 5′-CAGAAGTGGCAGACTCATTGGACTC -3′. GAPDH primers were as follows: Forward primer (FP): 5′-TGAAGGTCGGAGTCAACGGATT-3′ & Reverse primer (RP): 5′-CCTGGAAGATGGGTGATGGGATT -3′. The reaction mix contained 10 µL TransStart Green qPCR SuperMix (2×), 0.5 µL FP, 0.5 µL RP, 2 µL cDNA, 0.4 µL reference dye and up to 20 µL nuclease-free water. The thermal profile was holding stage (95°C/10 minutes), cycling stage that includes 45 cycle, each has denaturation (95°C/15 seconds), annealing (53°C/30 minutes) & extension (72°C/30 seconds).

C. Data analysis: According to stepOne software version 2.2.2., the data were produced as sigmoid shaped amplification plots. The control samples were used as calibrators, so their expression levels were set to 1. The relative quantities of human KLF2 mRNA were normalized against that of human GAPDH, so relative quantitation (RQ) was calculated by the equation $2^{-\Delta\Delta CT}$. Figure 1

3.2 | Statistical analysis

Data were analyzed using IBM SPSS software package version 20.0 (IBM Corp). Qualitative data were described using number and percent. The Kolmogorov-Smirnov test was used to verify the normality of distribution Quantitative data were described using range (minimum and maximum), mean ± standard deviation (SD), and median Inter Quartile Range (IQR). The used tests were chi-square test, Monte Carlo correction, F-test (ANOVA), Mann-Whitney test, Pearson’s coefficient, Student’s t test, and receiver operating characteristic curve (ROC).
## RESULTS

The mean value of KLF2 mRNA was statistically significantly lower in AV patients (0.61) than controls (1.0) ($P < .001$) Table 1. The mean value of KLF2 mRNA was statistically significantly lower in inflammatory acne group (grades III, IV, and V) than noninflammatory acne group (grades I and II) and highest in the control group ($P < .001$). On comparing inflammatory and noninflammatory acne groups ($P_1 < .001$), on comparing inflammatory acne and controls ($P_2 < .001$) However, on comparing noninflammatory acne and controls ($P_3 = .082$) Table 2.

There was statistically significant difference in KLF2 level as regards the Global Acne Severity Scale in inflammatory acne group ($P < .001$). The mean value of KLF2 was lowest in grade V

### TABLE 1
Comparison between acne patients and control subjects according to KLF2

<table>
<thead>
<tr>
<th>KLF2</th>
<th>Acne patients ($n=100$)</th>
<th>Control ($n=50$)</th>
<th>$t$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min.-Max.</td>
<td>0.15-1.08</td>
<td>0.88-1.17</td>
<td>10.099</td>
<td>&lt;.001*</td>
</tr>
<tr>
<td>Mean ± SD.</td>
<td>0.61 ± 0.37</td>
<td>1.0 ± 0.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>0.71 (0.20-0.96)</td>
<td>0.98 (0.93-1.09)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Statistically significant at $P \leq .05$.

Abbreviation: $t$, Student’s $t$ test.

### TABLE 2
Comparison between the three groups according to KLF2

<table>
<thead>
<tr>
<th>KLF2</th>
<th>Inflammatory acne ($n=50$)</th>
<th>Noninflammatory acne ($n=50$)</th>
<th>Control ($n=50$)</th>
<th>$F$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min.-Max.</td>
<td>0.15-0.76</td>
<td>0.67-1.08</td>
<td>0.88-1.17</td>
<td>578.639</td>
<td>&lt;.001*</td>
</tr>
<tr>
<td>Mean ± SD.</td>
<td>0.27 ± 0.16</td>
<td>0.95 ± 0.09</td>
<td>1.0 ± 0.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>0.20 (0.16-0.28)</td>
<td>0.96 (0.89-1.03)</td>
<td>0.98 (0.93-1.09)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: $F$ for ANOVA test, pairwise comparison bet each 2 groups were done using Post Hoc Test (Tukey).

$P$: $P$ value for comparing between the studied groups, $P_1$: $P$ value for comparing between inflammatory acne and noninflammatory acne, $P_2$: $P$ value for comparing between inflammatory acne and Control $P_3$: $P$ value for comparing between noninflammatory acne and Control.

*Statistically significant at $P \leq .05$.
(0.22) than in grade IV (0.29) and grade III (0.48), similarly in non-inflammatory acne group mean value of KLF2 was lower in grade II than in grade I but this difference was not statistically significant \( (P = .277) \). Despite that KLF2 mean value was higher in noninflammatory acne group than in inflammatory acne group Table 3 and Figure 2.

There was statistically significant difference in KLF2 level as regards pattern of healing in inflammatory acne group \( (P = .016) \). The mean value of KLF2 was lower in cases healed by scars (0.23) than cases healed by postinflammatory hyperpigmentation (PIH) (0.37), similarly KLF2 mean value was lower in cases healed by scars than those healed by PIH in noninflammatory acne group but this difference was not statistically significant \( (P = .314) \). Despite that KLF2 mean value was higher in noninflammatory acne group than in inflammatory acne group Table 3 and Figure 3.

There was statistically significant difference as regards effect of diet on KLF2 mRNA level in inflammatory acne group \( (P < .001) \). The mean value of KLF2 was lower in consuming fatty diet (0.20) than carbohydrate (0.4) and protein diet (0.35), also in noninflammatory acne group the mean value of KLF2 was lower in fatty consumers (0.93) than carbohydrate (0.95), and protein diet (0.98) but this difference was not statistically significant \( (P = .271) \) Table 3 and Figure 4.

The ROC curve showed that KLF2 level was statistically significant for diagnosis of inflammatory acne \( (P < .001) \) with 98% sensitivity and specificity. The probability of inflammatory acne among those with low KLF2 level is 98% (PPV 98) and that cutoff point of KLF2 for diagnosis of inflammatory acne is ≤0.67 relative quantitation (RQ) Table 4 and Figure 5.

5  | DISCUSSION

Inflammation plays a central role in AV pathogenesis together with androgens are prime orchestrators that lead to increased sebum production and a more inflammatory composition of sebaceous lipids.\(^1\) The \( P \) acne promotes mixed Th17/Th1 responses by inducing the concomitant secretion of IL-17A and IFN-\( \gamma \) from specific CD4 \( (\uparrow) \) T cells. Therefore, Th17-related cytokines potentiate acne pathogenesis.\(^6\) High levels of IL1, macrophages and CD4 cells in AV patients compared with apparently healthy subjects suggest that inflammation precedes hyperproliferation in acne pathogenesis.\(^5\) IL-1, through promoting comedone formation also may participate in inflammation and rupture of follicular canal.\(^7\)

Naive lymphocytes are quiescent until encounter specific antigens. KLF2 is expressed in resting lymphocytes (naive and memory cells) but is downregulated rapidly after lymphocyte activation, suggesting that it may be a quiescence factor.\(^8\) Although the physiological role of KLF2 in cell cycle control in lymphocytes is currently unclear, there is a growing evidence that KLFs regulate migration of inflammatory cells and lymphocytes during both normal homeostasis of immune system and inflammation.\(^9\)

Nuclear factor-kappa B (NF-\( \kappa B \)) is an important inducer of inflammation through transcription of several pro-inflammatory cytokines, chemokines, and adhesion molecules. KLF2 is a negative regulator of inflammation which reduces pro-inflammatory activity of NF-\( \kappa B \).\(^7\) It has been revealed that cellular levels of KLF2 are reduced in chronic inflammatory states. This shows the importance of KLF2 in the biological response to inflammation.\(^10\)

**TABLE 3** Relation between KLF2 and different parameters

<table>
<thead>
<tr>
<th>KLF2</th>
<th>Inflammatory acne</th>
<th>Noninflammatory acne</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean ± SD.</td>
</tr>
<tr>
<td><strong>Grading</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>II</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>III</td>
<td>7</td>
<td>0.48 ± 0.24</td>
</tr>
<tr>
<td>IV</td>
<td>11</td>
<td>0.29 ± 0.15</td>
</tr>
<tr>
<td>V</td>
<td>32</td>
<td>0.22 ± 0.10</td>
</tr>
<tr>
<td><strong>Healing</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PIH</td>
<td>15</td>
<td>0.37 ± 0.20</td>
</tr>
<tr>
<td>SCAR</td>
<td>35</td>
<td>0.23 ± 0.12</td>
</tr>
<tr>
<td><strong>Diet</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>13</td>
<td>0.40 ± 0.19</td>
</tr>
<tr>
<td>Fatty diet</td>
<td>30</td>
<td>0.20 ± 0.07</td>
</tr>
<tr>
<td>Protein diet</td>
<td>7</td>
<td>0.35 ± 0.22</td>
</tr>
</tbody>
</table>

Note: Pairwise comparison bet. each two groups were done using Post Hoc Test (Tukey).

Statistically significant at \( P \leq .05 \).

Abbreviations: #, Excluded from the association due to small number \( (n = 1) \); F, ANOVA test; \( P \), \( P \) value for comparing between CHO and Fat; \( P \), \( P \) value for comparing between CHO and Protein; \( P \), \( P \) value for comparing between Fat and Protein; \( t \), Student’s \( t \) test.
Out of all members of KLF family, KLF2 and KLF4 have been detected in vascular endothelium, lymphoid cells, and in skin, gut, kidney, and lung epithelium.\(^2^1\)

In this study, the mean value of KLF2 was lower in acne patients (0.61) than control group (1.0), lower in inflammatory acne group (grades III, IV, and V) than noninflammatory acne group (grades I and II) and highest in the control group (\(P < .001\)). Similar pattern of KLF2 was found by Das et al\(^1^0\) who observed that downregulation of KLF2 and concomitant upregulation of several inflammatory markers as IL-6, TNF-\(\alpha\), and MMPs (MMP1, 9, and 13) in patients with active rheumatoid arthritis established the inverse correlation between KLF2 levels and inflammatory cytokines expression. Wang et al\(^1^3\) found that KLF2 participates in the inflammatory response of ulcerative colitis by negatively regulating expression of IL6, IL-8, IL-10, and TNF-\(\alpha\) levels. These results were supported by Nayak et al\(^2^0\) who stated that deficiency of KLF2 modulates the in vivo response to acute (sepsis) and subacute (skin) inflammation and enhances pro-inflammatory cytokines expression.

Acne pathogenesis entails the development of acne lesions by induction of the secretion of IL1, IL6, IL8, and TNF-\(\alpha\).\(^2^2\) It is well established that the secretion of pro-inflammatory cytokines such as IL1, IL8, TNF-\(\alpha\), and MMPs contributes to the inflammatory nature of acne.\(^2\)

Decreased KLF2 enhances pro-inflammatory cytokines expression, vascular instability, interstitial fluid accumulation, and macrophage infiltration. So, its reduction enhances the process of acute inflammation through augmentation of pro-inflammatory gene expression and macrophage recruitment to the site of inflammation.\(^2^0\)

van Vliet et al\(^2^3\) reported that mammalian KLFs are known to perform critical functions in lipid metabolism and lipogenesis in adipose and nonadipose tissues. Also, Ling et al\(^2^4\) stated that KLF2 has a new function as it inhibits fat build-up.
TABLE 4 Receiver Operator Characteristics curve (ROC curve) for KLF2 mRNA to diagnose inflammatory acne

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cut off</th>
<th>AUC</th>
<th>P</th>
<th>95% C.I</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>KLF2 mRNA (RQ)</td>
<td>≤0.67</td>
<td>0.999</td>
<td>&lt;.001</td>
<td>0.995-1.0</td>
<td>98.0</td>
<td>98.0</td>
<td>98.0</td>
<td>98.0</td>
</tr>
</tbody>
</table>

Statistically significant at P ≤ .05.

Abbreviations: AUC, area under the curve; CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value; RQ, relative quantitation.

Sebocytes, in addition to producing sebum, link lipid metabolism with inflammation at a cellular level and hence greatly resemble adipocytes. Sebocyte and adipocyte share the expression of many adipogenic factors during their differentiation. KLF2 dramatically diminishes upon adipocyte differentiation as excess KLF2 prevents preadipocyte differentiation. Thus, KLF2 may have a role in sebocyte differentiation which may affect acne pathogenesis.

FoxO1 function is a key regulator in the pathogenesis of acne as FoxO1 suppresses lipid metabolism through suppression of the activity of PPARγ. FoxO1 deficiency results in spontaneous T-cell activation. FOXO1 can drive the induction of KLF2 in human T cells as FOXO1 binds the KLF2 promoter in vivo. Same result was found by Fathy et al who found that the mean value of TLR2 was significantly increased in acne patients and the expression was still higher among those with predominantly inflammatory lesions than those with predominantly noninflammatory lesion. Knockdown of KLF2 expression augments the activation of TLR2 through NFκB.

As regards Global Acne Severity scale, we found that the mean value of KLF2 was lower in grade V acne cases (0.22), grade IV (0.29), and grade III (0.48) than in noninflammatory acne group grade II (0.93) and grade I (0.96) (P < .277). These results were supported by Wang et al who stated that severe ulcerative colitis patients showed the lowest expression of KLF2, suggesting that KLF2 was negatively correlated with the inflammatory level, illustrated that KLF2 is linked to the severity because the level of cytokines IL6, IL8, IL10, and TNFα was higher in severe than mild ulcerative colitis patients. Also, Amr et al found that there was a significant association between PPARγ as a suppressor marker of inflammation and acne severity graded by global acne grading system (P < .001) revealing that the PPARγ is less common in patients with severe acne. Overexpression of KLF2 induces PPARs.

As regards the pattern of healing of acne lesions, we found that the mean value of KLF 2 was lower in cases healed by scarring (0.23) than in cases healed by post inflammatory hyper pigmentation (0.37) which denotes continuous process of inflammation in cases of scarring. Holland et al found that the inflammatory response has been implicated as an important component in the development of scar. Also, Saint-Jean et al found that absence of scars is associated with low expression of pro-inflammatory cytokines as TLR2, IL2, TNFα, and MMPs. Das et al found that overexpression of KLF2 inhibits the induction of pro-inflammatory cytokines such as IL2 and TNFα.

As regards the effect of diet on KLF2 mRNA expression, the level was significantly lower in acne patients on high fat than carbohydrate and protein diet; however, no literature is available concerning this finding.

6 | CONCLUSIONS

Acne pathogenesis involves many factors that cause inflammation and formation of different types of lesions with different degrees of severities. These factors include the alteration of the KLF2 level. We observed that KLF2 was downregulated in AV patients suggesting a role in the inflammatory acne pathogenesis negative relation was found between serum KLF2 level and AV severity and scar formation. This suggests that KLF2 could serve as a marker of inflammation and scaring in AV.

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CONFLICT OF INTEREST

The authors have declared no conflicting interests.

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