INTRODUCTION

Acne vulgaris is a disease of pilosebaceous units which results from the interplay between multiple factors. The sequence of events in acne pathogenesis is still not certain, but inflammation is strongly proposed as the early initial factor.\(^1\)

*Propionibacterium acnes* initiates and maintains the inflammatory process via different mechanisms including increasing the proinflammatory cytokines mainly interleukin-1 beta (IL-1\(\beta\)) release.\(^2\) Interleukin-1\(\beta\) can be released from keratinocytes, fibroblasts, and immune cells such as macrophages and mast cells. It can be involved in the etiopathogenesis of inflammatory and autoimmune disorders, as it has a regulatory effect on chemokine expression and T-cell extravasation. Moreover, it is directly implicated in tissue destruction.\(^3,4\)

C-reactive protein (CRP), one of the acute phase proteins, is the best indicator of systemic inflammation, as its level elevates rapidly in cases of inflammation, and its serum levels show no circadian changes across the day. IL-1, IL-6 and tumor necrosis factor alpha (TNF-\(\alpha\)) that are implicated in the pathogenesis of acne are also major inducers of CRP production by the liver. Thus, CRP levels could be elevated in acne if the amount of inflammation is high enough.\(^5\)

Recently, the possible value of saliva to monitor the overall health, to diagnose various oral or systemic disorders,\(^6\) and to monitor the therapeutic levels of different drugs to modify and individualize the
dose$^7$ has been proposed based on the accumulating data from multiple studies.

In laboratory setting, blood and urine are the most commonly used samples for most of the investigations. However, it seems that saliva has some advantages over them.$^5$ Collecting saliva samples is easy, noninvasive, and painless.$^7$ It is also more suitable for certain cases were collecting blood samples is difficult, for example, in pemphigus vulgaris patients in which extensive areas of skin are eroded.$^{10}$

The aims of this study were to assess the serum and salivary levels of IL-1$\beta$ and CRP in patients with active moderate-to-severe acne vulgaris and to evaluate the correlation between their serum and salivary levels. We aimed also to assess the correlation between serum and salivary levels of these markers and acne severity.

2 | SUBJECTS AND METHODS

This case–control study was conducted in the outpatient clinic of dermatology department. The study included 189 participants: 84 patients suffering from moderate-to-severe acne vulgaris, in addition to 105 apparently healthy, age-, sex-, and BMI-matched individuals as a control group. The study was approved by the local ethics committee on research involving human subjects of faculty of medicine. Informed consents were obtained from all participants before samples collection.

Subjects with acneiform eruptions, or history of topical or systemic therapy for acne vulgaris within 2 months before the study were excluded from this work. Smoking, pregnancy and lactation, infectious, inflammatory, autoimmune systemic or cutaneous diseases, as well as serious systemic illnesses, for example, liver, kidney, or cardiac disease were also among the exclusion criteria.$^{11,12}$ Subjects suffering from malignancy$^{13}$ and those using systemic drugs which may affect the inflammatory markers levels, for example, systemic steroids$^{14}$ were also excluded. A number of common (local) issues can substantially induce inflammation in the mouth and undoubtedly also contribute to a poor overall correlation between inflammations in saliva and blood. These issues include poor oral hygiene, gingivitis, periodontal disease, and mouth injury.$^{15}$ That is why all these conditions were also excluded from the study.

All of the enrolled patients were subjected to full history taking and complete general examination with emphasis on the body mass index (BMI). The skin was examined carefully to determine the types and distribution of acne lesions and to grade acne according to the global acne grading system (GAGS).$^{16}$

2.1 | Laboratory investigations

All participants were subjected to estimation of serum and salivary levels of C-reactive protein (CRP) and Interleukin-1 beta (IL-1$\beta$) using enzyme-linked immunosorbent assay (ELISA) technique.

2.1.1 | Blood sampling

Five ml of venous blood was withdrawn from each participant and put in serum-separating tube and left for 30 min until clotting, and was then centrifuged. The separated serum was stored at −2°C till assay.

2.1.2 | Salivary sampling

Two to ten ml of saliva was collected by unstimulated passive drool. Donor tilted his head forward, allowing saliva to pool on the floor of the mouth, then passed the saliva through saliva collection aid into a polypropylene vial. After collection, particulates were removed by centrifugation for 15 min at 1500 $g$, then were stored at −20°C till assay.

2.1.3 | Determination of serum and salivary levels of CRP

Serum and salivary levels of CRP were done by ELISA kits supplied by Sun red Biological Technology company, Shanghai, China. Catalogue No:201-12-1799. Standard range was 0.2–3.2 pg/L.

2.1.4 | Determination of serum and salivary levels of human IL-1$\beta$

Serum and salivary levels of IL-1$\beta$ were determined by ELISA technique using human IL-1 $\beta$ ELISA kits supplied by Sun red Biological Technology company, Shanghai, China. Catalogue No:201-12-0144. Analytical standard range was 300–4800 pg/L.

2.2 | Statistical analysis

The collected data were revised, coded, tabulated, and introduced to a PC using Statistical Package for Social Science (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.). Data were presented and suitable analysis was done according to the type of data obtained for each parameter.

Shapiro test was done to test the normality of data distribution. Descriptive statistics:

1. Mean, standard deviation (±SE or SE), median, minimum, and maximum for numerical data.
2. Frequency and percentage of non-numerical data.

Analytical statistics:

• Student T Test was used to assess the statistical significance of the difference between two study group means.
• Chi-Squared test was used to examine the relationship between two qualitative variables
• Correlation analysis: To assess the strength of association between two quantitative variables.
• N.B: $p$ is significant if $<0.05$ at the confidence interval of 95%.

3 RESULTS

The study included 84 moderate-to-severe acne vulgaris patients as well as 105 apparently healthy acne-free subjects as a control group. Patients and control subjects were matching regarding age ($p = 0.31$), gender ($p = 0.41$), and BMI ($p = 0.09$) (Table 1).

The mean age of acne vulgaris onset in the studied patients was 13.43 ± 3.6 years, and the mean duration was 4.7 ± 2.3 years. Patients’ examination revealed post acne scarring in 57 (67.8%) patients. The mean GAGs score in the current sample was 32.78 ± 6.9.

The serum and salivary levels of both CRP and IL-1β in the patients were significantly higher than the measured levels in the control subjects ($p < 0.001$) (Table 2).

Serum and salivary CRP levels showed significantly positive correlation with each other as well as with GAGS scores and serum IL-1β ($p < 0.001$). Serum IL-1β correlated significantly with salivary IL-1β and with GAGS scores ($p < 0.001$). The levels of IL-1β in saliva did not show significant correlation with GAGS scores or with serum and salivary CRP (Table 3). There was insignificant difference in the levels of the measured markers between patients with post acne scarring and those without (Table 4).

4 DISCUSSION

Saliva is an easily reachable fluid that can easily be collected by the patient. Many ingredients of saliva are derived from blood, so its constituents may reflect the systemic events. The main advantage of saliva testing is the easy, valid, and noninvasive sample collection procedure that is neither painful nor traumatic. This makes saliva serve as a potential alternative diagnostic fluid in infants, toddlers, youth, and adults.15,17

Analysis of saliva sample is a convenient means for assessment of physiological and pathological conditions and for evaluation of the concentration of drugs and different toxic and therapeutic substances.18 Saliva-based measurements also help in the early detection of certain diseases and monitoring the disease course. It was also found to be an alternative diagnostic fluid to serum for monitoring inflammation.19

Despite the wide use of saliva-based measurements, its use in dermatology is still limited. To the best of our knowledge, there are no available data about salivary markers in acne vulgaris patients. Therefore, the current study aimed at assessing the serum and salivary levels of IL-1β and CRP in patients with acne vulgaris and evaluating the relationship between their levels and the clinical aspects of the disease.

The present study detected a significantly higher serum levels of CRP in acne vulgaris patients when compared with the control group subjects. This result is in agreement with the findings of Mohammed et al.20 and El-Taweel et al.21

On the other hand, Namazi et al.22 did not detect an elevated serum CRP level in their acne vulgaris patients. They concluded that acne vulgaris, even in its severe grades, is not one of the conditions which induce significant inflammation at the systemic level. In fact, the nonsignificant difference between patients and controls in their study can be explained by the nature of the control group. They recruited the control patients from the blood donors who were not exactly matching the patients.

Karabay et al.23 also did not find any significant difference between CRP serum levels in patients and control groups. This may be as a result of the use of a quantitative CRP that cannot detect the minimal changes in CRP levels.

In the present study, the serum CRP levels showed a significant positive correlation with GAGS scores. This was in accordance with Mohammed et al.20 Namazi et al.22 found that the mean CRP levels in severe acne group was higher than that of the moderate acne group; however, the difference was insignificant. Using a different

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Demographic data and BMI of the studied groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variables</td>
<td>Control group $N = 105$</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>18.92 ± 2.81</td>
</tr>
<tr>
<td>Male</td>
<td></td>
</tr>
<tr>
<td>N (%)</td>
<td>55 (52.38)</td>
</tr>
<tr>
<td>Female</td>
<td></td>
</tr>
<tr>
<td>N (%)</td>
<td>50 (47.61)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>21.84 ± 2.12</td>
</tr>
</tbody>
</table>

Note: $p$ is significant if $<0.05$.
Abbreviations: BMI, Body mass index; SD, standard deviation; T, Student’s test; $\chi^2$, Chi-squared test.
acne severity assessment method (a modification of combined acne severity classification system) may harden the comparison between both studies.

Salivary CRP has been investigated and proved to have a diagnostic and prognostic value in a number of medical problems such as myocardial infarction and obesity. In the field of dermatology, salivary CRP in psoriasis patients was significantly elevated reflecting the inflammatory nature of the psoriasis.

The present study detected a significantly higher salivary level of CRP in acne patients compared with the control group. It seems also that salivary CRP reflects the degree of the disease severity as a significant positive correlation between salivary CRP and GAGS scores was reported. Although Namazi et al. suggested that the inflammatory reaction of acne might be a completely local reaction which does not affect the systemic levels of the inflammatory markers, the current results may change this concept. Hepatocytes are the source of CRP protein, which is produced in different inflammatory conditions. It appears in saliva as a component of gingival crevicular fluid, and it is not produced locally in the salivary glands. This proposes strongly the possibility to consider acne as a systemic inflammatory condition, especially the severe degrees.

In addition, there is a significant positive correlation between serum and salivary CRP levels in the acne patients. Serum and salivary CRP are likely to correlate because, unlike cytokines, CRP is synthesized primarily in the liver. Hence, there is no local production of CRP in the mouth and its most likely route into saliva is via blood.

A positive correlation was found between the CRP measured in blood and in saliva. Similar results were previously reported in other studies. All these findings propose salivary CRP as a sensitive less invasive inflammatory biomarker in acne vulgaris.

IL-1β is a proinflammatory cytokine, which is essential in mediating the role of P. acnes in initiating the inflammatory cascade involved in acne vulgaris development. Its tissue expression in inflammatory lesions of acne vulgaris is significantly elevated. In accordance with Trivedi et al., Sugisaki et al., and Askari et al., the

### Table 2: Serum and salivary CRP and IL-1β in the studied groups

<table>
<thead>
<tr>
<th>The studied markers</th>
<th>Control group N = 105</th>
<th>Patients group N = 84</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Serum CRP (mg/L)</td>
<td>0.46 ± 0.32</td>
<td>1.31 ± 0.85</td>
</tr>
<tr>
<td>Salivary CRP (mg/L)</td>
<td>0.92 ± 0.16</td>
<td>2.59 ± 1.17</td>
</tr>
<tr>
<td>Serum IL-1β (pg/L)</td>
<td>343.78 ± 276.3</td>
<td>886.65 ± 953.3</td>
</tr>
<tr>
<td>Salivary IL-1β (pg/L)</td>
<td>886.58 ± 574.71</td>
<td>2097.68 ± 2061.18</td>
</tr>
</tbody>
</table>

Note: p is significant if <0.05 and are indicated in bold.

Abbreviations: CRP, C-reactive protein; IL-1β, Interleukin-1 beta; L, liter; mg, milligram; pg, Pico gram; SD, Standard Deviation; t, Student test.

### Table 3: The correlation between the studied markers and variables

<table>
<thead>
<tr>
<th></th>
<th>Serum CRP</th>
<th>Salivary CRP</th>
<th>Serum IL-1β</th>
<th>Salivary IL-1β</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAGS scores</td>
<td>0.65</td>
<td>0.907</td>
<td>0.420</td>
<td>-0.1</td>
</tr>
<tr>
<td></td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.365</td>
</tr>
<tr>
<td>Serum CRP</td>
<td>-0.79</td>
<td>0.69</td>
<td>0.61</td>
<td>0.473</td>
</tr>
<tr>
<td></td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.067</td>
</tr>
<tr>
<td>Saliva CRP</td>
<td>-0.79</td>
<td>0.69</td>
<td>0.61</td>
<td>0.473</td>
</tr>
<tr>
<td></td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.067</td>
</tr>
<tr>
<td>Serum IL-1β</td>
<td>0.61</td>
<td>0.69</td>
<td>0.61</td>
<td>0.473</td>
</tr>
<tr>
<td></td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.067</td>
</tr>
<tr>
<td>Saliva IL-1β</td>
<td>0.473</td>
<td>0.473</td>
<td>0.473</td>
<td>0.473</td>
</tr>
<tr>
<td></td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.067</td>
</tr>
</tbody>
</table>

Note: p is significant if <0.05 and are indicated in bold.

Abbreviations: CRP, C-reactive protein; GAGS, Global acne grading system; IL-1β, Interleukin-1 beta; r, Pearson correlation coefficient.

### Table 4: Markers levels in patients with post acne scarring and those without

<table>
<thead>
<tr>
<th></th>
<th>Serum CRP</th>
<th>Salivary CRP</th>
<th>Serum IL-1β</th>
<th>Salivary IL-1β</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with post acne scarring (n = 57)</td>
<td>1.43 ± 0.9</td>
<td>2.67 ± 1.3</td>
<td>974.7 ± 1128</td>
<td>2324.36 ± 2483.6</td>
</tr>
<tr>
<td>Patients without post acne scarring (n = 27)</td>
<td>1.05 ± 0.7</td>
<td>2.41 ± 0.8</td>
<td>700.76 ± 377.5</td>
<td>1619.14 ± 299.6</td>
</tr>
<tr>
<td>p</td>
<td>0.6</td>
<td>0.33</td>
<td>0.22</td>
<td>0.14</td>
</tr>
</tbody>
</table>

Note: p is significant if <0.05 at confidence interval 95%.

Abbreviations: CRP, C-reactive protein; IL-1β, interleukin-1-beta.
The present study detected significantly higher serum levels of IL-1β in the patient's group.

In contrast, a previous study reported that acne patients have higher serum concentrations of IL-10, but not IL-1β compared with healthy volunteers. Acne patients who were recruited in that study were on treatment with isotretinoin, which may reduce IL-1β levels in serum of acne vulgaris patients with the decrease in sebum production.

In the present study, it was interesting to find that IL-1β levels in serum showed a significant positive correlation with GAGS scores. This result was in line with Mochtar et al. To the best of our knowledge, salivary IL-1β was not evaluated in dermatology except in psoriasis patients, where salivary levels were significantly elevated and the marker was considered a disease biomarker. The present study reports a significantly higher salivary IL-1β levels in acne patients. A significant positive correlation between serum and salivary IL-1β levels was found in the current acne patients' sample. Rilis et al. and La Fratta et al. also found a positive correlation between salivary and blood levels of the IL-1β in healthy adolescent girls and in stressed persons, respectively.

Significant positive correlations between serum and salivary CRP and serum IL-1β have been noticed in this study. Elevated serum concentrations of IL-1β have been found in patients with acne vulgaris, providing an etiology for the elevated CRP, as Interleukin IL-1 (proinflammatory cytokine) stimulates the liver to produce acute-phase proteins such as CRP.

Salivary IL-1β did not show significant correlation with GAGS scores or with serum and salivary CRP levels. In fact, IL-1β in saliva might be less accurate than serum IL-1β in reflecting the systemic events. Salivary IL-1β is derived from the circulation and can also be synthesized and released locally from acinar and ductal cells in the oral salivary glands. La Fratta et al. found that salivary IL-1β levels depend on the activity of salivary glands. This might explain the lack of significant correlation between IL-1β in saliva and the GAGS scores and CRP levels in serum and saliva.

The significant elevation in the studied markers in serum and saliva, besides significant positive correlation between serum and salivary CRP and serum IL-1β levels with GAGS scores, suggests these molecules to be biomarkers which can objectively be used in evaluating the degree of inflammation in acne patients. The changes in their levels with treatment could help an objective monitoring of the patients. Studying the effect of different anti-acne drugs on the levels of these markers is recommended. Moreover, the current study suggests an important role of IL-1β in acne pathogenesis. Targeting this molecule may be a promising novel anti-acne approach which needs to be studied.

This study proposes measuring salivary markers (CRP and IL-1β) as a sensitive and noninvasive tool to evaluate the inflammatory process in acne vulgaris patients. It paves the road to utilize saliva-based measures in different inflammatory conditions in the field of dermatology, especially in conditions where blood sample collection might be difficult such as in geriatric patients and children, or in patients with bullous diseases affecting large surface areas.

4.1 Limitations

The study included moderate-to-severe acne vulgaris only. Mild acne cases were not included.

5 CONCLUSION

The current study supports the emerging role of saliva as a valid noninvasive tool for monitoring inflammation and as a reliable and stress-free tool to evaluate cytokines and other inflammatory marker levels in acne vulgaris.

CONFLICT OF INTEREST

No conflict of interest to declare.

ETHICAL APPROVAL

The study was approved by the local ethics committee on research involving human subjects of faculty of medicine. Informed consents were obtained from all participants before samples collection.

DATA AVAILABILITY STATEMENT

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

ORCID

Rehab Mohammed Salem https://orcid.org/0000-0003-2805-1224

REFERENCES


34. Askari N, Ghazanfari T, Yaraee R, et al. Association between acne and serum pro-inflammatory cytokines (IL-1α, IL-1β, IL-1αR, IL-6, IL-8, IL-12 and RANTES) in mustard gas-exposed patients: sarashteh-iran cohort study. *Arch Iran Med*. 2017;20(2):86-91.


