Matrix metalloproteinase-9 in the blood of acne patients: The possible use of matrix metalloproteinase-9 as a biomarker of acne severity

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Introduction
Acne vulgaris is the most common disorder of the sebaceous gland. Microcomedones are the result of abnormal hyperproliferation of ductal keratinocytes and keratinization of acroinfundibular epithelium [1].

Triglycerides are considered as a main constituent of sebum. Keratinocytes, neutrophils, and microorganisms such as \textit{Propionibacterium acnes} are the other constituents. \textit{P. acnes} stimulates keratinocytes to produce microinflammatory cytokines such as interleukin-1α and tumor necrosis factor-α (TNF-α) [2]. Keratinocytes and some inflammatory cells such as mast cells, monocytes, and macrophages are important sources of TNF-α in the skin [3]. Human dermal fibroblast is considered as a source of TNF-α in response to external stimuli [4]. TNF-α plays a critical role in modulating matrix metalloproteinases (MMPs) activity in the dermis [5].

MMPs are a family of zinc-dependent endopeptidases having a critical role in skin biology during inflammatory matrix remodeling, neovascularization, wound healing, malignant transformation, and in pathological manifestations of diseases treated by retinoids as acne vulgaris [6].

The MMP gene family encodes four several proenzymes with common and distinctive structural and functional properties classified as collagenases, gelatinases, stromelysins, matrilysins, membrane type 1–6, and various others [7].

Several kinds of MMPs such as \textit{MMP}-1, \textit{MMP}-13, and \textit{MMP}-9 are found in the sebum of acne patients and some of them are reported in some researches to be upregulated in skin lesions of some acne patients [7]. \textit{MMP}-9 was detected in facial sebum of acne patients.
as seen by gelatin zymography and western blot analysis.

To the best of our knowledge, no studies have been carried out to investigate MMP-9 level in the serum of acne patients. Our study aimed to investigate the possible presence of MMP-9 in the blood of acne patients and to correlate its level with disease severity hoping to use it as a biomarker of acne severity.

Patients and methods
This case–control study was carried out on 45 patients with acne vulgaris and 20 normal controls matched for age and sex selected from the Dermatology Outpatient Clinic, Menoufia University Hospital. The study took place from June 2018 to January 2019. Acne grading was performed using a simple system taking into account the predominant lesions present.

According to physical and dermatologic examination, this study included the following groups: group 1 includes 15 patients with mild acne; group 2 includes 15 patients with moderate acne; and group 3 includes 15 patients with severe acne.

The study was approved by the ethics committee of medical research of the Faculty of Medicine, Menoufia University. Written informed consent was obtained from all participants before the study.

Exclusion criteria
(1) Patients under isotretinoin therapy
(2) Patients with hyperproliferative skin disorders such as psoriasis and lichen planus
(3) Patients with bullous diseases, dermal fibrosis, tumors, and metastasis
(4) Patients with chronic ulcers.

All participants were subjected to the following: Full history taking, complete general examination, dermatological examination, laboratory investigations including measurements of MMP-9 serum levels by enzyme-linked immune sorbent assay (ELISA) technique.

Sample collection
From each participant, 3 ml of venous blood was withdrawn under complete aseptic conditions from the cubital vein. The samples were dispensed in plain tubes and were allowed to clot at room temperature, followed by centrifugation at 3000 rpm for 15 min and the serum was kept frozen at −70°C until analysis.

Measurements of MMP-9 level
Serum MMP-9 level was measured by the ELISA technique. ELISA Kit Donghu Hi-Tech Development Area, Wuhan, Hubei, P.R.C, China (catalogue no.: 201-12-0937).

Principles of the assay
The kit uses a double-antibody sandwich ELISA to assay the level of human MMP-9 in the samples. Add MMP-9 to the monoclonal antibody enzyme well which is precoated with human MMP-9 monoclonal antibody, this is followed by incubation; then add MMP-9 antibodies labeled with biotin and combined with streptavidin–HRP to form an immune complex; then carry out incubation and washing again to remove the uncombined enzyme. Then add chromogen solution A, B; the color of the liquid changes into blue, and under the effect of acid, the color finally becomes yellow. The chroma of color and the concentration of the human substance MMP-9 of the sample were positively correlated.

Statistical analysis
Unpaired Student’s t-test was used to compare between two groups in quantitative data.

Analysis of variance (ANOVA) tests were done according to the computer program SPSS for Windows (SPSS Inc., Chicago, Illinois, USA). ANOVA test was used for comparison among different items in the same group in quantitative data.

Tukey’s test was used to determine which means among a set of means differ from the rest.

P value levels of significance are as follows:
(1) $P > 0.05$, nonsignificant
(2) $P \leq 0.05$, significant
(3) $P < 0.001$, highly significant.

Results
This study included a total of 45 patients with acne vulgaris, 22 (48.89%) men and 23 (51.11%) women, their age ranged from 17 to 25 years with a mean ± SD of 20.067 ± 1.935 and 20 controls matched for age and sex, 13 (65.00%) men and seven (35.00%) women, their age ranged from 17 to 25 with a mean ± SD of 20.650 ± 2.758. The acne patients were divided into three groups: mild, moderate, and severe; each group included 15 patients. Regarding MMP-9 level in acne patients, the mean ± SD was 32.896 ± 28.926, while the level of controls was with a mean ± SD of 11.818 ± 7.580. There was a significant difference in the level of MMP-9
between patients and controls with a $P$ value of 0.002. Using ANOVA test, the MMP-9 level in the group with mild acne was found to have a mean ± SD of 13.689 ± 3.675 while in moderate acne group, mean ± SD of 23.679 ± 7.022, and that for the severe group had a mean ± SD of 61.320 ± 34.857. Tukey’s test showed a significant difference between mild and severe group of less than 0.001 also between moderate and severe of less than 0.001. There was an association between MMP-9 blood levels and the number of regions with inflammatory nodules and pustules but not with scars. The MMP-9 level did not correlate with duration of illness, age, or sex of the patients (Tables 1 and 2).

### Discussion

Cellular culture studies and advanced immunology techniques have been increasingly demonstrated that acne is a disease of innate immunity and previously known pathogenic factors that likely interact with various immune mechanisms to promote acne pathogenesis. While it has classically been thought that alteration in keratinization leads to inflammatory events, it is now believed that these inflammatory events are preceded by interleukin-1 secretion [8].

The role of *P. acnes* is not only to activate innate immune cells but also to activate keratinocytes, macrophages, monocytes, and sebocytes with the expression of cytokines, chemokines, and MMPs [9]. Sebum has a direct inflammatory effect as well as an indirect regulatory effect of downstream inflammatory pathways [8].

Matrix metalloproteinases are extracellular matrix-degrading enzymes that interact and form a lytic cascade for remodeling of extracellular matrix. Activation of NFkB and activator protein-1 leads to the upregulation of MMPs like MMP-1, MMP-8, MMP-9, and MMP-13. Each of these are involved in the degradation of collagen [10].

Prolonged cycles of upregulation of MMPs and subsequent procollagen synthesis are prolonged in acne-scar prone patients. An imbalance in the ratio of MMPs to their tissue inhibitors results in atrophic or hypertrophic scars [11].

Many studies have investigated the expression of MMPs and their inhibitors in facial sebum of acne patients. Gelatin zymography and Western blot analysis showed the presence of MMP-9 in the sebum of acne patients and its level was decreased following per os or topical treatment with isotretinoin [7].

Another study showed that *P. acnes* extract caused an increase in TNF-α expression in human dermal fibroblast which in turn induces MMP-2 expression [12]. Other studies reported the expression of MMP-9 via the same role of *P. acnes* [13]. Under in-vitro conditions, *P acnes* was found to induce expression of MMP-9 from human keratinocytes [14].

To the best of our knowledge, the expression of MMP-9 was studied only in sebum and tissue cultures but not in the blood of acne patients. Our study aimed to detect whether MMP-9 levels will be elevated in the blood of acne patients as well as correlating its level with disease severity, so it can be used as a blood biomarker for acne vulgaris. In our study, MMP-9 blood level recorded significant elevation in patients with acne compared with the control group. The level of this gelatinase is significantly elevated in severe acne patients compared with moderate cases and also significantly upregulated in the blood of severe cases compared with mild cases. An important finding in our study is the association found between high levels of MMP-9 and the number of facial regions affected by inflammatory pustules and nodules but not with scars. Low levels of MMP-9 are found in hypertrophic and keloid scars causing lack and/or late apoptotic death of myofibroblasts, hence prolonging fibrinogenesis [11].

### Table 1 Matrix metalloproteinase-9 levels in controls and patients

<table>
<thead>
<tr>
<th>Groups</th>
<th>MMP-9 level</th>
<th>$t$-Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Mean±SD</td>
</tr>
<tr>
<td>Acne</td>
<td>9.11-110.87</td>
<td>32.896±28.926</td>
</tr>
<tr>
<td>Control</td>
<td>3.111-26.35</td>
<td>11.818±7.580</td>
</tr>
</tbody>
</table>

$t$-Test, unpaired Student’s $t$-test. MMP-9; matrix metalloproteinase-9. *$P$=0.002, statistically significant difference.

### Table 2 Matrix metalloproteinase-9 levels in different groups of acne patients and controls

<table>
<thead>
<tr>
<th>Acne severity</th>
<th>MMP-9 level</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Mean±SD</td>
</tr>
<tr>
<td>Mild</td>
<td>9.11-21.122</td>
<td>13.689±3.675</td>
</tr>
<tr>
<td>Moderate</td>
<td>12.11-36.206</td>
<td>23.679±7.022</td>
</tr>
<tr>
<td>Severe</td>
<td>15.818-110.87</td>
<td>61.320±34.857</td>
</tr>
<tr>
<td>Control</td>
<td>3.111-26.35</td>
<td>11.818±7.580</td>
</tr>
</tbody>
</table>

Tukey’s test

<table>
<thead>
<tr>
<th>Mild and moderate</th>
<th>Mild and severe</th>
<th>Mild and control</th>
<th>Moderate and severe</th>
<th>Moderate and control</th>
<th>Severe and control</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.414</td>
<td>$&lt;0.001^*$</td>
<td>0.990</td>
<td>$&lt;0.001^*$</td>
<td>0.211</td>
<td>$&lt;0.001^*$</td>
</tr>
</tbody>
</table>

There is a statistically significant difference between severe, mild, and moderate cases and also with normal. ANOVA, analysis of variance; $F$, frequency; MMP-9, matrix metalloproteinase-9. *$P$=0.001.
Our study recorded a negative correlation between MMP-9 blood levels and age, sex and disease duration. We cannot detect other studies dealing with MMP-9 blood level in acne patients but studies on other gelatinases as MMP-8 in acne inversa were done. These studies agreed with our results as they demonstrated that high lesional MMP-8 in acne inversa was accompanied by elevated MMP-8 blood levels and these levels were positively correlated with disease activity and recorded a strong positive association between its level and TNF-α in the blood [15].

**Conclusion**

We suggest that MMP-9 blood quantification should be incorporated in future clinical trials to be used as a biomarker of acne severity because the currently used methods are subjective and time consuming and usually incorrect results are detected by different physicians and centers.

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Nil.

**Conflicts of interest**

There are no conflicts of interest.

**References**