

# Calprotectin may be positively associated with the severity of acne vulgaris

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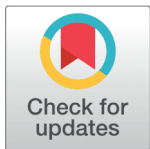
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## OPEN ACCESS

## ABSTRACT

**Background and objective:** Acne vulgaris (AV) is a common skin disease of sebaceous hair follicles. Many factors are associated with the occurrence and severity of acne, while the exact etiology remains incompletely understood. The current study was aimed to investigate the association between the severity of acne and serum zinc, copper, and calprotectin.

**Methods:** Fifty patients with AV were recruited in the study as well as 25 healthy age and sex-matched individuals as controls. The acne severity was classified into mild (n=21), moderate (n=16), and severe acne (n=14) according to the global acne grading system (GAGS). Serum levels of zinc, acne and calprotectin were evaluated by enzyme-linked immunosorbent assay (ELISA). The gained data were analyzed using GraphPad Prism software.

**Results:** Insignificant difference was found in zinc and copper levels between controls and AV patients, except in severe AV, where the patients displayed significant elevation in serum copper level ( $p < 0.05$ ) as compared to that of mild AV. The calprotectin concentration was significantly higher ( $p < 0.001$ ) in all AV patients, when compared with healthy subjects, which was positively correlated with the disease severity. No gender difference was noted for all measured biomarkers.

**Conclusions:** Our study suggests a possible association between calprotectin and acne inflammation, which requires validation in large-scale studies.

**Keywords** acne vulgaris, calprotectin, inflammatory, copper, zinc

## INTRODUCTION

Acne is a common skin disease, ranking as the eighth most prevalent disease globally, with an estimated worldwide prevalence of 9.4%.<sup>1,2</sup> The severity of acne can range from

very mild such as physiologic acne to very severe in the form of acne conglobata.<sup>3</sup> The exact etiology is yet to be elucidated. Involvement of life style, genetic and environmental factors has been demonstrated to influence the pathogenesis,<sup>4-6</sup> in which interactions between hormones, bacteria (such as *Propionibacterium/Cutibacterium acnes* and *Staphylococcus epidermidis*), follicular differentiation and inflammation play a major role.<sup>7,8</sup>

Trace elements obtained daily from diet are necessary for normal cell functions and stabilization of cellular structure. As consequence, trace element deficiencies may activate alternative pathways and causes diseases.<sup>9</sup> Nutritional status has been suspected to be associated with acne,<sup>4</sup> in particular zinc and copper,<sup>10-12</sup> which was controversially discussed in the literature.<sup>13-15</sup>

Inflammation is widely reported in the progression and severity of acne.<sup>16,17</sup> The proinflammatory mediators upregulated in skin of AV may include E-selectin, vascular adhesion molecule-1, interleukin-1, integrin, defensin-2, interleukin-1 $\alpha$ , CD3+ and CD4+ T cells and macrophages.<sup>18-22</sup> Calprotectin is a calcium-binding intracellular protein, which have inflammatory cytokine activity and are made of at least two different chains, calgranulin A and B.<sup>23</sup> Calprotectin constitutes more than 50% of the neutrophil cytosolic protein content.<sup>24</sup> Overexpression of calprotectin in the keratinocytes was revealed in different inflammatory dermatoses.<sup>23</sup> However, the role of calprotectin in acne remains incompletely studied.

The current study was aimed to evaluate zinc, copper and calprotectin in sera of patients with AV and their correlations with the severity of acne.

## MATERIALS AND METHODS

### Study participants

Fifty patients with AV were recruited in this cross-sectional study as well as 25 healthy age and sex-matched individuals as controls. All the patients and healthy subjects were students at the Technical Institute of Baquba (Baqubah, Iraq) and they were randomly asked to participate. The global acne grading system (GAGS) was used to classify acne severity,<sup>25</sup> including mild (n=21), moderate (n=16), and severe AV (n=14), accordingly. The volunteers with acute or chronic diseases, malnutrition, malabsorption, or anemia were excluded, as well as pregnant and lactating women and those who underwent medical treatment or received dietary supplements. Written informed consent was obtained from each participant before enrollment. Body mass index (BMI) was calculated individually by using the following equation:

$$BMI = \frac{Weight (Kg)}{[Height (m)]^2}$$

Five milliliters of venous blood were obtained from each participant, collected on serum-separator gel tube and centrifuged after the samples clot. The gained sera were preserved at -20°C until the day of biochemical analysis.

## Biochemical analysis

Enzyme-linked immunosorbent sorbent assay (ELISA) was used to investigate concentrations of zinc, copper (Abcam, Japan) and calprotectin (G-Biosciences, United States) in the serum of each participant. The manufacturers' instructions were followed.

## Statistical analysis

GraphPad Prism (United States) was used to analyze the statistical data. To compare the means of two groups, unpaired sample t-test or Mann Whitney's test was used as appropriate. For three groups and more, comparisons were performed by one way ANOVA or Kruskal-Wallis test as appropriate. A *p*-value of 0.05 or less was considered statistically significant.

## RESULTS AND DISCUSSION

The Table 1 illustrates sociodemographic characteristics of the study participants. It shows no significant differences among groups in gender, age, and BMI.

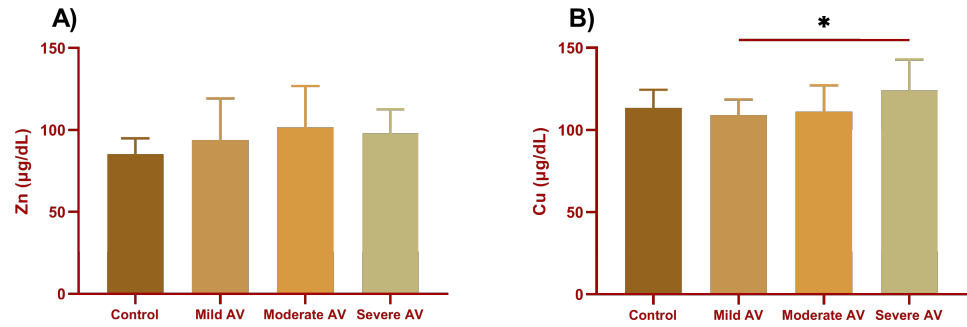
**Table 1** Baseline characteristics of the study participants.

Groups Items	Control	Mild AV	Moderate AV	Severe AV	<i>P</i>
N	25	21	16	14	-
Gender (M/F)	11/14	12/9	8/8	8/6	0.79
Age (years)	22.92±3.30	21.62±3.23	23.00±1.41	23.71±3.51	0.22
BMI (Kg/m <sup>2</sup> )	23.67±1.45	24.28±1.51	23.73±1.34	23.73±1.30	0.51

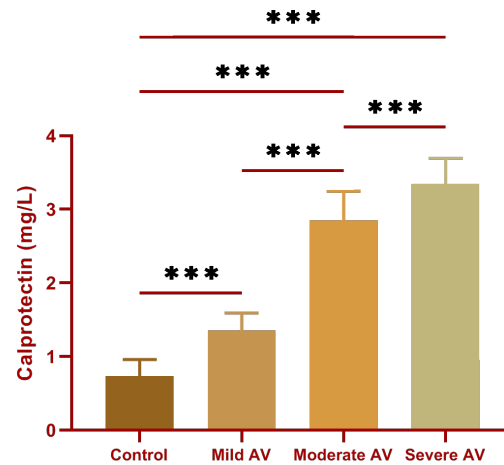
AV= acne vulgaris, M= male, F= female, BMI= body mass index.

The zinc and copper findings are presented in Figure 1. The mean zinc concentration in each AV subgroup was insignificantly higher than that of healthy controls (control: 85.20±9.68, mild AV: 93.98±25.29, moderate AV: 101.6±25.35, severe AV: 98.05±14.58; *p*=0.053). The copper concentrations in mild and moderate AV were lower than that of control group, without statistical significance (control: 113.5±11.11, mild AV: 109.1±9.519, moderate AV: 111.1±16.15, severe AV: 124.1±18.84; *p*= 0.627, 0.831, 0.085, respectively). However, among AV patients, copper level was significantly higher in severe than in mild AV (*p*<0.05).

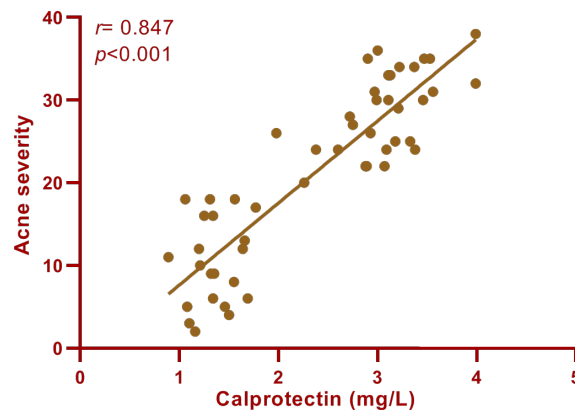
Serum calprotectin revealed significant elevation (*p*<0.001) in AV groups compared to non-acne participants (control: 0.73±0.22, mild AV: 1.35±0.23, moderate AV: 2.85±0.39, severe AV: 3.34±0.34) (Figure 2). Moreover, serum calprotectin level of AV groups was positively associated with the severity of acne by Spearman correlation analysis (*r*=0.847, *p*<0.001; Figure 3).



**Figure 1** Serum zinc and copper levels of acne patients and healthy controls. A) Serum zinc levels. B) Serum copper levels. \* denotes statistically significant alteration between mild and severe AV ( $p < 0.05$ ). AV= Acne vulgaris.

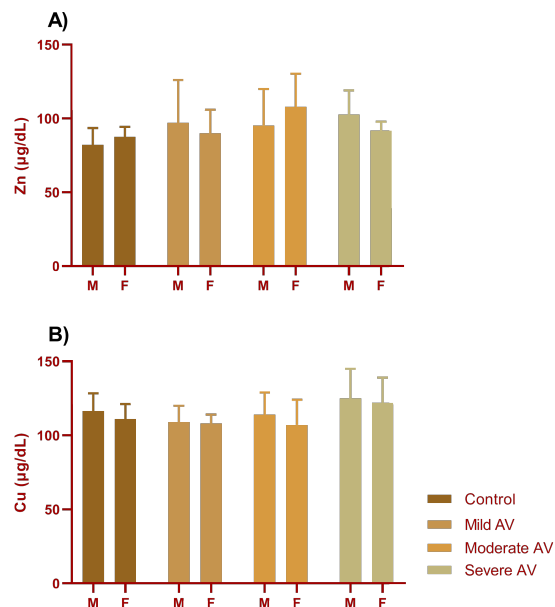


**Figure 2** Serum calprotectin of acne patients and healthy subjects.\*\*\* denotes highly statistical difference in means ( $p < 0.001$ ). AV= Acne vulgaris.

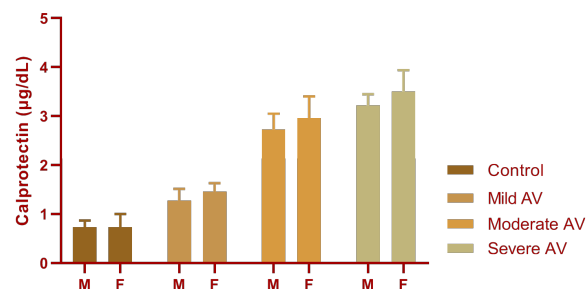


**Figure 3** Spearman correlation analysis between calprotectin and acne severity. Acne's severity axis represents GAGS scores.

The gender influence on the tested biomarkers was also assessed, but without significant changes (Figures 4 and 5).



**Figure 4** Serum levels of zinc and copper in acne patients and healthy individuals based on gender. M= male, F= female, AV= acne vulgaris. (T- or Mann Whitney' s U test were used)



**Figure 5** Serum level calprotectin in acne patients and healthy individuals based on gender. M= male, F= female, AV= acne vulgaris.

## DISCUSSION

Seborrhea, altered sebaceous duct keratinization, *Propionibacterium/Cutibacterium acnes* colonization, and inflammation have all been implicated in the pathophysiology of acne. Androgens, insulins, and insulin-like growth factor-1 are major contributory hormones. Hypersensitivity to androgen and inflammatory cytokines regulated by the innate immune system can aggravate the underlying condition.<sup>26</sup>

Calprotectin released by keratinocytes, phagocytes, monocytes, granulocytes and vascular cells is recognized by toll-like receptors (TLRs) to induce inflammatory response.<sup>27,28</sup> Our results indicated a high calprotectin concentration might be associated with the inflammatory responses of acne, which is in line with the data from previous studies.<sup>29,30</sup> Higher calprotectin was also evidenced in many other inflammatory diseases such as rheumatoid arthritis, psoriatic arthritis, juvenile idiopathic arthritis, neonatal sepsis, and multiple sclerosis.<sup>31,32</sup> Calprotectin can protect against inflammation-induced skin carcinogenesis.<sup>33</sup>

Calprotectin is positively associated with insulin resistance, while androgens (dehydroepiandrosterone sulfate and testosterone) and calprotectin together are elevated in inflammatory disease.<sup>34</sup> Neutrophils can hold microbial infection control by phagocytosis and release of anti-inflammatory molecules,<sup>35</sup> whereas about 50% of the cytoplasmic protein content of neutrophils is comprised of calprotectin.<sup>24,36,37</sup> Thus, the calprotectin upregulation in AV may be resulted from the inflammatory response.

Our results did not support the association between the zinc and copper levels and acne.<sup>14</sup> However, higher copper level in severe than in mild acne was seen in the current study, which might be the result of chronic inflammatory response. A previous study showed that serum copper level could be higher in chronic diseases.<sup>38</sup>

The small sample size and the cross-sectional structure are the major limitations of the current study. GAGS is a simple but not comprehensive scale system in evaluation of acne. Post-adolescent acne was the focus of our study. Large-scale studies in longitudinal observation of acne in different age groups are required to validate our preliminary results.

## CONCLUSIONS

This study showed calprotectin might be a promising biomarker to evaluate the acne severity and response to treatment. Further research is needed to elucidate the underlying mechanisms.

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## DECLARATIONS

### Authors' contributions

Experimental part, drafting the original manuscript, data analysis and interpretation: SAA, ZSA, HAA. Reviewing the draft: WC, FA, HA. All authors read and approved the final version of the manuscript before publication.

## Conflict of interest

The authors declare no conflict of interest.

## Ethical approval and consent to participate

All procedures performed in the study involving human participants were in accordance with the ethical standards of the Institutional Research Committee and with the 1964 Helsinki Declaration and its later amendments. Written informed consent was obtained from each participant before participation.

## Data availability

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

## Funding resources

No external fund was received.

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