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Serum Levels of Osteoprotegerin in Patients with Acne Vulgaris

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Abstract
Background: *Acne vulgaris (AV) is a common chronic inflammatory skin disease involving pilosebaceous follicles characterized by comedones, papules, pustules, nodules, and sometimes scars.*
Aim of the Work: *The aim of this study is to evaluate the serum levels of osteoprotegerin in patients with acne vulgaris and assess its relation to the severity of the disease.*
Methods: *This case-control study was conducted in the outpatient Clinic of Dermatology and Andrology, Department of Benha University. The study included 80 participants; 60 patients suffering from acne vulgaris, in addition to 20 apparently healthy, age and sex matched individuals as a control group. Determination of serum level of osteoprotegerin was done by ELISA technique.*
Results: *The mean serum level of OPG in patients group (124.390±5.741) was significantly higher than that in the control group (47.394±29.332) as p<0.001. At a Cutoff point >48.4, Serum OPG had 95% Sensitivity and 70% Specificity and 88.9% accuracy to differentiate between Patients and Controls.*
Conclusion: *Osteoprotegerin may play a role in acne pathogenesis.*
Keywords: *Acne vulgaris. osteoprotegerin, case-control.*

Introduction

Acne vulgaris (AV) is a common chronic inflammatory skin disease involving pilosebaceous follicles characterized by comedones, papules, pustules, nodules, and sometimes scars[(1)]. Acne can present as noninflammatory lesions, inflammatory lesions, or a mixture of both, affecting mostly the face but also the back and the chest[(2)]. The disease commonly begins in adolescence and is perceived as teenage disease; however, it often persists into adulthood[(3)].

Osteoprotegerin (OPG), also known as Osteoclastogenesis inhibitory factor (OCIF), or tumor necrosis factor receptor super family member 11B (TNFRSF11B), is a protein that in humans is encoded by the TNFRSF11Bgene. Osteoprotegerin is a cytokine receptor, and a member of the tumor necrosis factor (TNF) receptor super family[(4)]. Osteoprotegerin is a decoy receptor for the receptor activator of nuclear factor kappa B ligand (RANKL). By binding RANKL, OPG prevents RANK-mediated nuclear factor kappa B (NF-κB) activation which is a central and rapid acting transcription factor for immune-re1ated genes, and a key regulator of inflammation, innate immunity, and cell survival and differentiation[(5)]. Osteoprotegerin is produced

by a variety of tissues including the cardiovascular system (heart, arteries, veins), lung, kidney, intestine, and bone, as we11 as hematopoietic and immune cells[6]. The expression and production of the protein is modulated by various cytokines, peptides, hormones and drugs. Cytokines, including TNF-α, interleukin (IL1)-1α, IL1-18, transforming growth factor (TGF)-β, bone morphogenetic proteins, and steroid hormones such as 17 β-estradiol are known to up-regulate OPG mRNA levels[7]. RANKL may promote peripheral immune tolerance. For instance, it has been reported that RANKL-expressing keratinocytes in inflamed skin trigger epidermal dendritic cells to induce a Treg phenotype in infiltrating T cells[8].

The aim of this study was to evaluate the serum levels of osteoprotegerin in patients with acne vulgaris and assess its relation to the severity of the disease.

Subjects and Methods

I. **Subjects:** This case-control study was conducted in Outpatient Clinic of Dermatology and Andrology Department of Benha University. The study included 80 participants; 60 patients suffering from acne vulgaris, in addition to 20 apparently healthy, age and sex matched individuals as control group. Written informed consents were obtained from all participants. The study was approved by the local Ethics Committee on Research involving human subjects of Benha Faculty of Medicine.

Exclusion Criteria: Subjects with any of the following conditions were excluded from the studyinfectious, inflammatory or autoimmune cutaneous or systemic diseases, malignancy, pregnancy and lactation and liver or kidney disease.

II. **Methods**

Patients under study were subjected to full history taking, general examination including BMI[9] and dermatological clinical examination to detect acne lesions and to determine their types, distribution and grading. The grading of acne lesions was assessed according to the global acne grading system (GAGS)[10]. Laboratory investigation: Determination of serum level of osteoprotegerin was done by ELISA technique.

Blood Sampling

Five mls of venous blood sample were collected from each participant after overnight fasting under complete aseptic condition. Collected sample was put on plain plastic tube and allowed to clot, then centrifuged at 3000 r.p.m for 10 min.The separated serum was stored in a1iquot at -20 °C for subsequent determination of osteoprotegerin using ELISA technique**.**

Determination of serum level of osteoprotegerin: Serum level of osteoprotegerin was done by ELISA kits supp1ied by Shanghai Sun red Biologica1 Technology company, Shanghai, China.

Test Principle: The microtiter plate provided in this kit has been pre-coated with an antibody specific to osteoprotegerin. Standards and samp1es were added to the appropriate microtiter plate wells with a biotin-conjugated polyclona1 antibody preparation specific for osteoprotegerin and Avidin conjugated to Horseradish Peroxidase (HRP) was added to each micro plate well and incubated. Then a TMB substrate solution was added to each well. Only those wells that contain, biotin-conjugated antibody and enzyme-conjugated Avidin was exhibited a change in color. The enzyme-substrate reaction was terminated by the addition of a sulphuric acid solution and the color change was measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of in the samples was then determined by comparing the O.D. of the samples to the standard curve.

Assay Procedure: All reagents were allowed to reach room temperature. All the reagents were

mixed thoroughly by gently swirling before pipetting.

1. 100 µl of Standard, Blank, or Sample per well were added. It was covered with the Plate sealer then incubated for 2 hours at 37℃.
2. The liquid of each well was removed. 100 µl of Detection Reagent Aworking solution were added to each well. It was covered with the Plate sealer then incubated for 1 hour at 37℃. Detection Reagent A working solution appeared cloudy. It was Warmed to room temperature and mixed gently until solution was appeared uniform.
3. Each well was aspirated and washed, the process was repeated three times for a tota1 of three washes. Each well was washed by filling with Wash Buffer (approximately 400 µl) using a squirt bottle, multi-channel pipette, manifold dispenser or auto washer. After the last wash, all the remaining Wash Buffer were removed by aspirating or decanting. The plate was inverted and blotted it against clean paper towels.
4. 100 µl of Detection Reagent B working solution were added to each well. It was covered with a new Plate sealer then incubated for 1 hour at 37℃.
5. The aspiration/wash was repeated as in step 4.
6. 90 µl of Substrate Solution were added to each well. It was covered with a new Plate sealer then incubated within 15-30 minutes at 37℃and Protected from light.
7. 50 µl of Stop solution were added to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
8. The optical density of each well was determined at once, using a microplate reader set to 450 nm

Results

- ✓ This study included 80 participants, 60 acne patients and 20 subjects control. There was insignificant difference between patients and controls regarding age, BMI and sex (p= 0.515, 0.386 and 0.242 respectively) **(Table 1)**
- ✓ The GAGS score was 21.950± 8.903, positive post acne scar was found in 38.33% of the studied patients and the post inflammatory hyperpigmentation was found in 93.33% **(Table 2).**
- ✓ Then mean serum level of OPG in patients group was significantly higher than that in the control group **(Table 3)**
- ✓ There was significant correlation between serum OPG levels and the relation to smoking **(Graph 1).**
- ✓ There was significant correlation between serum OPG levels and GAGS scorewhile there was negative correlation between serum OPG levels and body mass index **(Table 4).**
- ✓ The best cutoff point of osteoprotegerin for the prediction of acne cases was more than 48.4 and the corresponding sensitivity was 95%, specificity was 70%, positive predictive value was 90.5%, negative predictive value was 82.4% and the accuracy was 88.9% **(Table 5)**

Table (1): Sociodemographic data of the studied groups

		Groups				T-Test or Chi-square	
		Patients		Controls		T	P-value
Age	Mean ±SD	18.833±2.924		19.350±3.438		-0.655	0.515
BMI	Mean ±SD	24.637±4.005		25.570±4.560		-0.871	0.386
Sex	Male	6	10.00	4	20.00	1.371	0.242
	Female	54	90.00	16	80.00		

BMI: body mass index, SD: standard deviation, P<0.05 is significant.

Table (2): Clinical findings in the patients group

Clinical aspects	N	%
GAGS. (Mean± SD)	21.950± 8.903	
Positive Post Acne Scar	23	38.33
Positive PIH	56	93.33

GAGs: global acne grading system, SD: standard deviation, PIH: post inflammatory hyperpigmentation.

Table (3): Serum levels of osteoprotegerin in the studied groups

		Groups		T-Test	
		Patients	Controls	t	P-value
Serum OPG	Mean ±SD	124.390±75.741	47.394±29.332	4.421	<0.001*

BMI: body mass index, SD: standard deviation, P<0.05 is significant, OPG: osteoprotegerin.

Graph (1): Variation in serum OPG levels between smoking and negative smoking

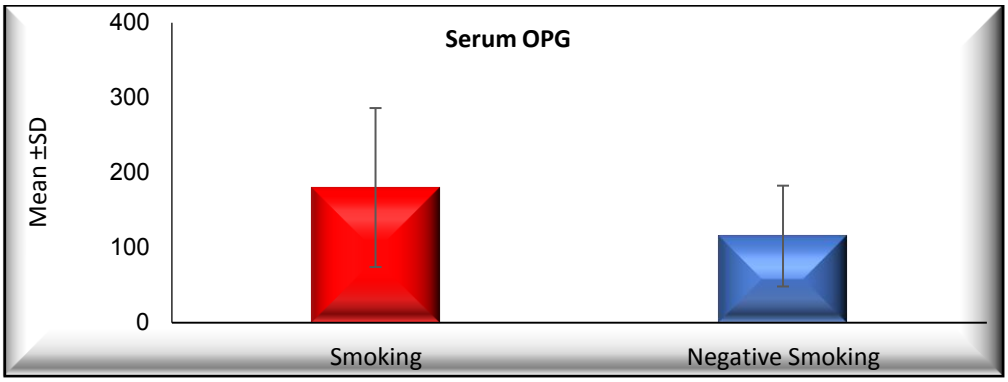

Table (4): Correlation between serum osteoprotegrin and studied variab1es

Correlations		
	Serum OPG	
	R	P-value
BMI	0.059	0.656
GAGS.	0.362	0.005*

BMI: body mass index, OPG: osteoprotegerin, , P<0.05 is significant,

Table (5): Diagnostic performance of osteoprotegerin for acne

ROC curve between Patients and Controls						
	Cutoff	Sensitivity %	Specificity %	PPV %	NPV %	Accuracy %
Serum OPG	>48.4	95.00	70.00	90.5	82.4	88.9

OPG: osteoprotegerin, ROC: receiver operating characteristic, PPV: positive predictive value, NPV: negative predictive value.

Discussion

Acne vulgaris (AV) is the most common cutaneous disorder seen in ambulatory dermatology practice among the pediatric and adult populations[11]. Osteoprotegerin (OPG) is a glycoprotein first described in 1997[4]. It is produced in many different tissues, including bone, vasculature, heart, lung, kidney, and placenta, and also circulates in plasma, although the concentration here is much lower than in bone and arterial tissue[12].

Besides, OPG has other biological functions, including anti-inflammatory actions, such as an anti-apoptotic effect resulting from the binding of TNF-related apoptosis-inducing ligand (TRAIL) with a consequent inhibition of the apoptosis process of susceptible cells. Interestingly, endothelial cells are one of the sites in which the

anti-apoptotic effects of OPG have been demonstrated, suggesting a protective vascular role of the latter. Therefore, OPG has, apart from the aforementioned effects on osteoclastogenesis, other functions in vascular processes and immune responses that may be relevant to the pathogenesis of acne vulgaris[13].

The aim of this study was to evaluate the serum levels of osteoprotegerin in acne vulgaris and assess its relation to the severity of the disease. In this study, the mean age of case was 18.833±2.924 versus 19.350±3.438 in control group. also, the mean value of BMI was 24.637±4.005 and 25.570±4.560 in case and control group respectively. and the majority of cases and control was female (90% &80% respectively). with insignificant difference between patients and controls regarding age, BMI and sex (p= 0.515, 0.386 and 0.242 respectively). These data coincide with the findings that AV is a common disease occurring throughout the adolescence as in this period the puberty associated with hormonal changes. In particular, androgens increase and cause sebaceous glands in the skin to enlarge and produce more sebum leading to acne lesions.This comes in accordance with the conclusion of acne review which reported that nearly 90% of teenagers in have acne [2].

Rizvi and Chaudry[14] reported that sufficient evidence for a positive relationship between acne and age. This becomes more frequently occurring disease after the time of puberty.

In accordance with this study, El-Tonsy et al., 2018[15] showed the mean of their patients' age was (23.36 ± 4.84 years.), there were 67 females and 33 males with female predominance (67%) with no significant difference between the genders (P = 0.20).

Moreover, females are more affected (45.7%) than the males (29.6%) which was also reported by Tallab, 2004[16]. It might be due to hormonal changes, which are supposed to facilitate the initiation of premenstrual acne[16]. Also, the 1,002 peoples enrolled in Zahra Ghodsi et al.[17] study were 499 boys (49.8%) and 503 girls (50.2%) aged between 12 and 20 years (mean±SD 16±0.9 years)[17]. Another study by Ismail and Mohammed-Ali,[18] who found the same in his study on included 510 patients (173 males and 337 females) with a male: female ratio of 0.41:1 [18].

In contrast, a study by Tan and Bhate,[19] revealed that male patients were more frequently affected, particularly with more severe forms of acne.

In current study, 55% of patients had positive family history, 66% had positive relation to stress and 93.33% had positive relation to sun exposure and 86.67% with positive re1ation to diet. 13.33% only had positive relation to smoking. This observation was explained by a genetic component with increasing rates of acne among twins and first-degree relatives. Also, stress is a cause of increased androgen secretion, increased sebum production, and reducing the immune status.

This was in agreement with Bagatin et al., 2014[20] who found about 50% of his patients reported family history for acne in mother or father but it was of no statistical significant association regarding family history.

Regarding sun exposure, there is a1so a report of Europeans deve1oping a type of acne after a beach holiday (acne Mallorca). A similar phenomenon has been observed in India and is locally referred to as 'Goa acne' [21]. Regarding dietary habit, it was established from the data that consumption of high glycemic index diet and high glycemic load exacerbates risk of acne occurrence according to Dikshit et al.[22].

Also, Qidwai et al.[23] reported that, diets with a high glycemic index, such as white bread, sugar, popcorn, and white rice, are promptly absorbed, conducting increased blood glucose levels. It has been shown to enhance sebum production, stimulate adrenal androgen synthesis, and increase androgen bioavailability and facilitating cell proliferation, all of which play a role in the pathogenesis of acne.

Bowe et al.[24] reported that the occurrence of acne is lower in rural and non-industrialized areas than

in Western populations. It is believed that it may be a result of differences between glycemic loads of diets of both populations.

Alanazi et al.[25] found post inflammatory hyper-pigmentation and scarring were present in 11.6% and 8.7% respectively, and scar was absent in most of cases (91.3%). However, El-Hamd et al.[26] found that 75% had post acne scarring, but this difference might be explained by the fact that our study is a community based study and the latter study is a hospital based study.

This study was the first trial was conducted to explore the role of OPG in pathogenesis of acne. Our idea was based on its relationship with obesity and insulin resistance which explored in many trials. As the insulin resistance and hyperglycemia, fat & sugar rich food were implicated in pathogenesis of acne. Also, the role of OPG in PCO (acne was one of clinical symptoms in it) was cleared in recent trials

A published data by Holecki et al.[27] showed that obesity and insulin resistance result in a decrease in serum OPG concentrations. Also, Yin et al.[28] revealed the adipose tissue, predominantly visceral adipose tissue (VAT), produces and secretes a variety of bioactive adipocytokines. However, VAT accumulation induces adipocytes dysfunction, including oversecretion of interleukin-6, tumor necrosis factor-α, plasminogen activator inhibitor-1 and visfatin, and hyposecretion of adiponectin, which were supposed to be involved in the pathogenesis of insulin resistance and abnormal glucose metabolism.

Also, Avington et al.[29] reported that increased circulating OPG levels have also found in association with the presence and severity of coronary artery disease, peripheral artery disease and stroke, suggesting that OPG may serve as a biomarker of established atherosclerosis in humans.

A study by Genc et al.[30] included 40 healthy volunteers and 40 psoriasis patients. The mean serum osteoprotegerin level was 279.3 ± 24.8 pg/ml in the psoriasis group and 273.9 ± 26.3 pg/ml in the healthy control group ($p > 0.05$).

Also, Lappin et al., 2007[31] support our finding, as they reported that, cigarette smoker patients tended to have lower serum concentrations of OPG than non- smoker patients, there were significant differences in the median serum concentration of OPG (smokers 23.76 pM, non- smokers 59.28 pM) and the ratio of serum concentrations of OPG. Concentrations of OPG in the smoker patients also had a statistically significant negative correlation with tobacco consumption.

Conclusion

The difference of osteoprotegerin levels was significantly differentiated between the patients with acne and the healthy control group. OPG may play a role in acne vulgaris pathogenesis.

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