

Evaluation Of Serum Levels Of Ghrelin And Obestatin In Acne Vulgaris Patients And Their Relations To Disease Severity

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Abstract

Background Acne vulgaris is a chronic, inflammatory disease of the pilosebaceous unit. Acne is a skin disorder associated with multifactorial pathogenesis. Obestatin and ghrelin are largely produced throughout the GI tract (e.g., stomach, pancreas and duodenum) with predominant expression in the gastric mucosa. Ghrelin produced in deep hair follicles and sebaceous glands may fold pathogenesis of acne vulgaris and cause acne in humans. **Aim** The aim of this study is to evaluate serum level of Ghrelin and Obestatin in Acne Vulgaris patients and to correlate between level of Ghrelin and Obestatin and disease severity. **Patients and Methods** The study is a prospective case control study that was conducted on patients with acne vulgaris aged between 15-30 years. Forty patients (15males and 25 females) with acne vulgaris and forty normal healthy control (21 males and 19 females) who matched the cases group as regard age and sex were included in this work. They were selected from the Dermatology Outpatient Clinic, Mansoura University Hospital. **Results** Serum ghrelin was significantly lower in the Acne Vulgaris group with mean \pm SD (585.98 \pm 21.08) when compared to the control group with mean \pm SD (1139.2 \pm 84.22), $p < 0.001$. Serum obestatin was non significantly lower in Acne Vulgaris group with mean \pm SD (202.5 \pm 14.72) when compared to the control group with mean \pm SD (265.7 \pm 33.14), $p > 0.05$. Serum ghrelin decreased significantly with increased severity ($p < 0.001$). Serum obestatin decreased non significantly with increased severity ($p > 0.05$). Serum ghrelin showed significant positive correlation with serum obestatin among all studied patients. **Conclusion** serum ghrelin and obestatin levels decreased in acne vulgaris patients and serum ghrelin showed significant negative correlation with severity.

Key words: ghrelin, obestatin, acne vulgaris.

INTRODUCTION

Acne vulgaris is a chronic inflammatory disease of the upper pilosebaceous unit, the name given to the hair follicle (HF) and its associated sebaceous gland (SG) [1] and it is a skin condition that is well characterized clinically [2].

Acne may present as comedones (i.e., whiteheads and blackheads), pustules, nodules, cysts or papules. Scars or facial deformities affect up to 20% of teenagers. Irritation, itching and local pain are physical symptoms that can contribute to reduced quality of life [3].

Acne vulgaris is the eighth most common disease globally, affecting over 0.5 billion people [4].

The number of prevalent cases of acne vulgaris globally was 231.2 million in 2019 [5].

Acne is a skin disorder associated with multifactorial pathogenesis, which can be divided into essential four stages: sebum overproduction, follicular hyper keratinization, proliferation of propionibacterium. acne, and immune response [6].

There are many studies that associate hormones with acne vulgaris (AV). Androgens are among the most studied hormones known to affect cutaneous biology. The roles of androgens in sebum production are yet to be known. However, many studies performed demonstrated that human pilosebaceous unit can be under the effect of several

hormones consisting amino acid, oligopeptide, polypeptide, protein, glycoprotein, lipid and phospholipid structures besides the androgens [7].

Ghrelin is an orexigenic hormone which is primarily secreted by the stomach and duodenum. It stimulates appetite and food intake along with regulation of growth hormone and insulin secretion; glucose and lipid metabolism; gastrointestinal motility; blood pressure, heart rate, and neurogenesis [8]. It is reported that ghrelin is highly expressed in the hair follicles and in the sebaceous glands of the skin [9].

Based on bioinformatic searches Zhang et al.,2005 identified obestatin, a 23-amino-acid amidated peptide encoded by the ghrelin precursor (prepro-ghrelin). The term obestatin originated from the Latin verb 'obedere', meaning 'to devour', and 'statin', denoting suppression. Indeed, obestatin was initially claimed to behave as a physiological opponent of ghrelin, inhibiting either ghrelin orexigenic action or the stimulatory effect on GH secretion [10].

The studies conducted on ghrelin and ghrelin gene products: obestatin and des-acyl ghrelin have shown that these hormones have roles in many diseases as well as their treatment. The studies on these hormones and skin diseases are very few and the relation of these hormones and the skin is intriguing [11].

The aim of the present study was to determine serum levels of ghrelin and obestatin in acne vulgaris patients and compare them to healthy control and to investigate the relationship between ghrelin &obestatin and acne severity.

Patients and Methods

This was a prospective case control study that was carried out on patients with acne vulgaris attending the Dermatology outpatient clinic of Mansoura University Hospital within a duration of one year, after obtaining the approval of Institutional Research Board (IRB) (MS.21.01.1334). Informed consent was obtained from each participant.

Forty patients with acne vulgaris and 40 age- sex matched controls were included in this study.

All patients diagnosed with acne vulgaris aged between 15- 30 years were included in the study.

The patients were excluded if their body mass index (BMI) were > 25, on any diet regimen, pregnant females, having endocrinologic disease such as diabetes, hyper- or hypothyroidism, having history of cancer or using alcohol.

Clinical evaluation

All patients underwent the following: Comprehensive medical history, including age, sex, occupation, marital status, special habits, previous disorders, also comprehensive general examination: all applicable clinical data, such as height, weight, BMI were collected and a thorough dermatological examination was performed on the skin, hair, nails, and oral mucosa.

The severity of the disease was evaluated according to the Global Acne Grading System (GAGS) [12]. GAGS consider six locations of the face and chest/upper back with a factor for each location based on surface area (forehead = 2, Right cheek = 2, Left Cheek =2, Nose = 1, Chin = 1, Chest and Upper back = 3), distribution and density of pilosebaceous units. Each region would be given a score depending on the type of lesions (No lesion =0, One comedone = 1, Papule=2, One pustule = 3, One nodule = 4) and the sum of scores multiplied by the factors (Local score = Factor × Grade from 0 to 4), the sum of local scores gives the global score (0–52). The severity is graded as mild if the score was 1–18, moderate with scores form 19–30, severe with scores form 31–38, and as very severe if the score is more than 38.

Laboratory investigations

Serum samples were obtained via venepuncture. The samples are left for 15 min at 37°C, then centrifuged for 15 min at 3000 RPM, after which the clear supernatant is collected in Eppendorf and kept at -20°C for further use.

Serum ghrelin and obestatin levels were measured using the Enzyme Linked Immunosorbent Assay (ELISA) method with commercial kits.

Statistical analysis

The collected data was revised, coded, and tabulated using Statistical package for Social Science (**IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.**). Data were presented and suitable analysis was done according to the type of data obtained for each parameter.

Results

The current study included 40 patients with acne vulgaris, their mean age was (19.08±3.43 years) and 40 control persons who were selected to be matched in age, with mean age of (19.58±3.63 years). **Table (1).**

This study included 15(37.5%) male and 25 (62.5) female in the acne vulgaris groups and 21(52.5%) male and 19 (47.5%) female in the control group. **Table (1).**

Table (1) Comparison of demographic data of acne Vulgaris cases and control groups

	Acne Vulgaris n = 40		Control n = 40		Test (p)
	No.	%	No.	%	
Gender					
Male	15	37.5	21	52.5	X ² =1.818 p=0.178
Female	25	62.5	19	47.5	
Age (years)					
Mean ± SD.	19.08 ± 3.43		19.58 ± 3.63		t- student=0.633 p=0.528
Median (Min. – Max.)	18 (15 – 28.0)		18 (15 – 28)		

The duration of the disease ranged between (0.08 to 10 years) with mean± SD (1.95 ± 0.32)., and the score ranged between (9 to 53) with mean ±SD (24.55 ± 1.82). **Table (2).**

The severity of disease included 35%(n=14) mild, 35%(n=14) moderate, 17.5%(n=7) severe, and 12.5%(n=5) very severe.

Table (2).

Table (2) Clinical parameters of the disease in acne Vulgaris cases

			AV N=40	
Duration of disease (years)	Mean ± SD.		1.95 ± 0.32	
	Median (Min. – Max.)		1.48 (0.08 – 10.0)	
Score	Mean ± SD.		24.55 ± 1.82	
	Median (Min. – Max.)		22.50 (9.0 – 53.0)	
Severity	Mild	N (%)	14	(35.0)
	Moderate	N (%)	14	(35.0)
	Severe	N (%)	7	(17.5)
	Very severe	N (%)	5	(12.5)

This study showed that serum ghrelin was significantly lower in the Acne Vulgaris group ranged between (268 to 932.8), with mean ±SD (585.98 ± 21.08) when compared to the control group ranged between (527 to 2928.6), with mean ±SD (1139.2 ± 84.22), p<0.001. **Table (3).**

This study showed that serum obestatin was non significantly lower in Acne Vulgaris group ranged between (112 to 405.7), with mean ± SD (202.5 ± 14.72) when compared to the control group ranged between (88.77 to 1099.4), with mean ±SD (265.7± 33.14), p>0.05). **Table (3).**

Table (3) Comparison of serum Ghrelin and obestatin among acne Vulgaris cases and control groups:

	Acne Vulgaris (n = 40)	Control (n = 40)	Test (p)
Serum Ghrelin (pg/ml)			
Min. – Max.	268.0 – 932.8	527.0 – 2928.6	Mann-Whitney U= 1465.0 p<0.001*
Mean ± SD	585.98 ± 21.08	1139.2 ± 84.22	
Median	600.6	1015.4	

Serum obestatin (pg/ml)			
Min. – Max.	112.0 – 405.7	88.77 – 1099.4	Mann-Whitney U=919.50 p=0.250
Mean ± SD	202.5 ± 14.72	265.7 ± 33.14	
Median	146.5	191.3	

Table (4) showed that: serum ghrelin decreased significantly with increased severity ($p < 0.001$). The highest level was re- corded in those with mild acne with mean (687.4±25.2) followed by those with moderate, severe, and very severe acne with mean (603.5±20.2, 515.1±21.6, 352.3±37.4, respectively).

Table (4) Association between serum ghrelin and acne Vulgaris severity

Severity	AV (N=40)				Test (p)
	No.	Serum Ghrelin (pg/ml)			
		Mean ± SD.	Median	Min. – Max.	
Mild	14	687.4±25.2	667.5	582.7-932.8	Kruskal-Wallis Test=26.7 p<0.001
Moderate	14	603.5±20.2	594.9	463.8-782.7	
Severe	7	515.1±21.6	508	459.4-602.4	
Very severe	5	352.3±37.4	311.7	268-456.7	

Table (5) showed that: serum obestatin decreased with increased severity, but no association was found between both parameters ($p > 0.05$).

Table (5) Association between serum obestatin and acne vulgaris severity

Severity	AV (N=40)				Test (p)
	No.	Serum obestatin (pg/ml)			
		Mean ± SD.	Median	Min. – Max.	
Mild	14	215.7±24.6	181	123.8-356.8	Kruskal-Wallis Test=1.367 p=0.713
Moderate	14	195.6±23.5	148.9	120.6-355.8	
Severe	7	166.7±25	142.3	112-300.8	
Very severe	5	134.6±64.8	135.7	121.8-405.7	

Receiver operating characteristic (ROC) curve of serum ghrelin and Obestatin was conducted for discrimination between healthy persons and patients with acne vulgaris. Serum ghrelin showed high accuracy AUC (AUC=0.911, $p < 0.001$) as diagnostic ability for AV, and serum Obestatin showed low accuracy AUC (AUC=0.575, $p > 0.250$) as diagnostic ability for AV. **Fig (1)**.

Combining ghrelin and obestatin, increased the area under ROC curve (AUC), and thereafter, the diagnostic ability of obestatin ($p < 0.001$), but not ghrelin ($p > 0.905$). **Fig (1)**.

Table (6) showed that at best cut off value of ghrelin (<720.8), sensitivity was 90%, specificity was 87.5%, PPV was 87.8%, NPV was 89.7%, accuracy was 88.8%. While at best cut off value of obestatin (<172.03), sensitivity was 60%, specificity was 57.5%, PPV was 58.5%, NPV was 58.97%, accuracy was 58.75%.

Table (6) Validity of Serum ghrelin and obestatin for discrimination between AV cases and control groups

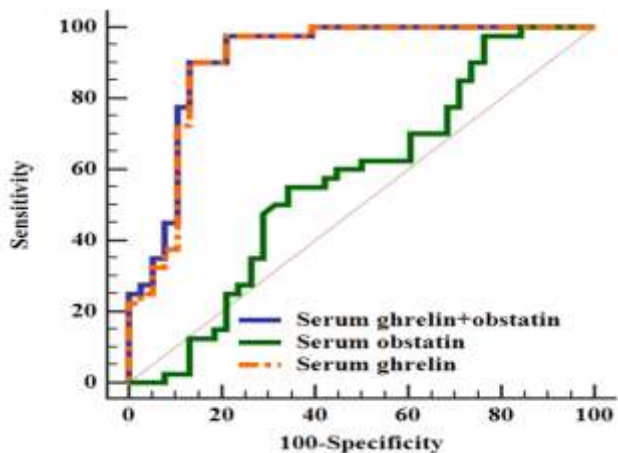


Figure (1) ROC Curve for Serum ghrelin and obestatin for discrimination between acne Vulgaris cases and control groups

Fig. (2) showed that serum ghrelin showed significant negative correlation with disease severity.

	Serum ghrelin	Serum obestatin	Serum ghrelin and obestatin
AUC	0.911	0.575	0.916
95% CI	0.849 – 0.982	0.447 – 0.702	0.833 - 0.979
P1	<0.001*	0.250	<0.001*
Cut off	<720.8	<172.03	-
Sensitivity (%)	90	60	90
Specificity (%)	87.5	57.5	87.5
PPV (%)	87.8	58.54	87.8
NPV (%)	89.7	58.97	89.7
Accuracy (%)	88.8	58.75	88.8
P2	-	<0.001	0.905
P3	-	-	<0.001

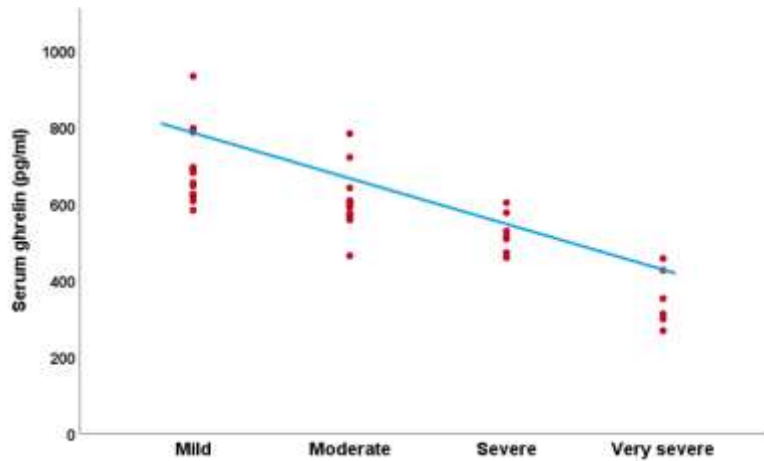


Figure (2) Correlation between serum ghrelin and severity

Fig. (3) showed that serum ghrelin showed significant positive correlation with serum obestatin among all studied groups.

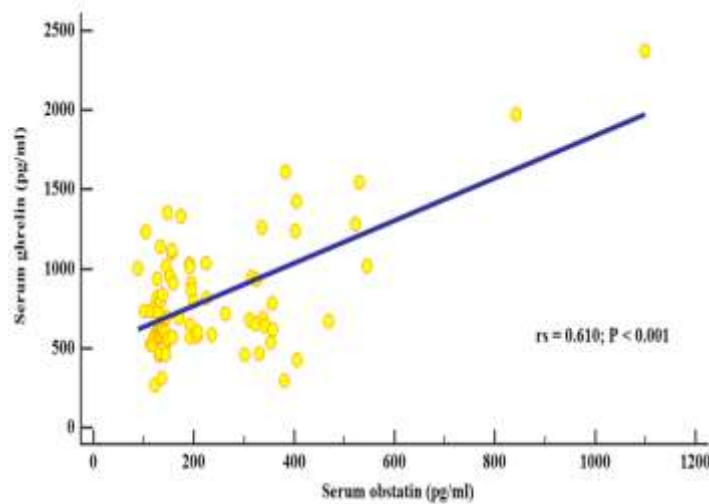


Figure (3) Correlation between Serum Ghrelin and Serum obestatin among all studied groups:

Logistic regression analysis was conducted for the prediction of AV susceptibility and showed that lower serum ghrelin was associated with the risk of AV susceptibility.

Ordinal regression analysis was conducted for the prediction of disease severity among acne Vulgaris patients and showed that serum Ghrelin was considered as risk predictor of disease severity.

Discussion

Acne is one of the most common and widespread skin conditions, affecting the health as much as patients' quality of life [13].

Recently, they reviewed that epidemiological risk factors influencing acne presentation and severity include demographic, genetic and hormonal, dietary, and personal factors; familial history, age, BMI, and skin type stood out as the strongest factors [14].

Recently, a few hormones coded by the same gene have been discovered (obestatin, des-acyl ghrelin and acylated ghrelin that is also known as a bioactive peptide). The mRNA of ghrelin has been detected in almost all tissues [11].

The studies conducted on ghrelin and ghrelin gene products: obestatin and des-acyl ghrelin have shown that these hormones have roles in many diseases as well as their treatment [11].

The current study included 40 patients with acne vulgaris, their mean age was (19.08±3.43 years) and 40 control persons who were selected to be matched in age, with mean age of (19.58±3.63 years).

In this study, severity of the disease included 35%(n=14) mild, 35%(n=14) moderate, 17.5%(n=7) severe, and 12.5%(n=5) very severe

In this study, Serum ghrelin was significantly lower in the Acne Vulgaris group ranged between (268 to 932.8), with mean ± SD (585.98 ± 21.08) when compared to the control group ranged between (527 to 2928.6), with mean ±SD (1139.2 ± 84.22), $p < 0.001$ and this was in accordance with *Kanat et al.*, [11] and *Abdel Rahman & El Esaway*, [8].

While serum obestatin was non significantly lower in Acne Vulgaris group ranging between (112 to 405.7), with mean ±SD (202.5 ± 14.72) when compared to the control group ranging between (88.77 to 1099.4), with mean ±SD (265.7± 33.14), $p > 0.05$, and this was agreement with *Kanat et al.*, [11].

Regarding validity of Serum ghrelin and obestatin for discrimination between AV group and control groups, we found that Serum ghrelin showed high accuracy AUC (AUC=0.911, $p < 0.001$) as diagnostic ability for AV, and serum Obestatin showed low accuracy AUC (AUC=0.575, $p > 0.05$) as diagnostic ability for AV.

In our study, at best cut off value of ghrelin (720.8), sensitivity was 90%, specificity was 87.5%, PPV was 87.8%, NPV was 89.7%, accuracy was 88.8%, while *Abdel Rahman & El Esaway*, [8] illustrated that ghrelin hormone can significantly ($P = 0.003$) predict post adolescent acne at cut off value ≤ 1012.5 with 73.9% sensitivity, 72% specificity, 72.5% PPV, and 73.5% NPV. Its accuracy was 73%, AUC (95%CI) = 0.721 (0.59-0.80).

While in our study, at best cut off value of obestatin (172), sensitivity was 60%, specificity was 57.5%, PPV was 58.5%, NPV was 58.97%, accuracy was 58.75%.

Our study showed that combining ghrelin and obestatin increased the area under ROC curve (AUC).

Regarding association between serum ghrelin and acne vulgaris severity, our study revealed that serum ghrelin decreased significantly with increased severity ($p < 0.001$), and the same results were reported by *Abdel Rahman & El Esaway*, [8] who found that acne severity had a significant effect on ghrelin's level.

Regarding association between serum obestatin and acne vulgaris severity, we found that serum obestatin decreased with increased severity, but no association was found between both parameters ($p > 0.05$), the highest level was recorded in those with mild acne with mean (215.7±24) followed by those with moderate, severe, and very severe acne with mean (195.6±23.5, 166.7±25, 134.6±64.8 , respectively). In another study conducted by *Kanat et al.*, [11] levels of obestatin were : mild :76.19 ±48.47, moderate: 84.28 ±54.12, sever: 64.64 ±33.35, with p value: 0.17.

Our study reported that serum ghrelin showed significant positive correlation with serum obestatin among all studied groups.

Logistic regression analysis was conducted for the prediction of AV susceptibility and revealed that lower serum ghrelin was associated with the risk of AV susceptibility.

Ordinal regression analysis was conducted for the prediction of disease severity among acne Vulgaris patients and showed that serum Ghrelin was considered as risk predictor of disease severity.

Conclusion and Recommendations

We can conclude that: Apart from the increase in androgen sensitivity, sebum secretion, microorganisms and inflammation that play roles in the etiology of AV, also decreased ghrelin and obestatin levels play roles in the pathogenesis, serum ghrelin showed significant negative correlation with severity and there is positive correlation between serum ghrelin and obestatin.

We recommend that replacement of these hormones (ghrelin and obestatin) to physiologic concentrations may contribute to the treatment of acne.

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