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Evaluation of Serum Level of Histone Deacetylase 1 Enzyme in Patients with Acne Vulgaris

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ABSTRACT
Background: Acne vulgaris (AV) is a highly prevalent skin inflammatory disorder. Histone modifications are common epigenetic processes, which happen secondary to environmental conditions. One of the many biological pathways' epigenetic regulation mechanisms is histone acetylation and deacetylation.
Objective: To evaluate the serum levels of histone deacetylase 1 (HDAC1) enzyme in AV cases and to examine their correlation with AV severity.
Patients and Methods: A case-control study was conducted on 90 participants: 45 patients with acne vulgaris and 45 healthy controls (HC). Global acne grading system (GAGS) was calculated. Then, serum HDAC1 level was measured by using ELISA.
Results: Cases showed significantly higher HDAC1 level when compared to control group. HDAC1 among case group showed a significant gradual increase in mild, moderate, severe, and very severe grades. Moreover, positive significant correlation with the score of acne vulgaris was demonstrated ($p<0.001$).
Conclusion: Human histone deacetylases1 (HDAC1) appears to be dysregulated in cases with AV. This could suggest a possible therapeutic opportunity for HDAC inhibitors for AV management.
Keywords: Acne Vulgaris, Histone Deacetylase 1, Histone Acetyltransferases, Global Acne Grading System.

INTRODUCTION

Acne vulgaris (AV) is a global inflammatory skin disease affecting the pilosebaceous follicles. More than 85% of teenagers complain from acne, which could last into adulthood [(1)]. The unique lesions could be classified as inflammatory (such as papules, pustules, nodules, and cysts) or non-inflammatory (open/black and closed/white comedone), which might be accompanied by scar formation [(2)].

Acne has a complex cause that involves multiple factors, including androgen-mediated sebum generation, follicular keratinization, inflammatory reactions, and Propionibacterium acnes (P. acnes) colonizing pilosebaceous follicles [(3)].

Genetic factors also involved in pathogenesis of acne [(4)].

Epigenetic modifications are key regulatory mechanisms of gene expression and differentiation in various cells which include keratinocytes. Histone acetylation is another well-known modification. Such process is regulated by histone acetyltransferases (HATs) and histone deacetylases (HDACs). Acetylation is accompanied by transcriptional stimulation, while deacetylation has been demonstrated to be accompanied by gene repression [(5)].

Histone deacetylase 1 (HDAC1) is a member of class I HDACs, which remove the acetyl group from histones and different proteins. Of note, both HDAC1 and HDAC2 have essential functions in the hair follicle and epidermal homeostasis. They are needed for normal hair development and cycling [(6)].

Propionibacterium acnes were demonstrated to trigger inflammation by suppression of histone deacetylase activities [(7)].

However, the exact contribution of epigenetic modifications to the pathogenesis of acne is not yet clear. So, the aim of the current study was to explain the potential role of epigenetic dysregulation in the development of AV by measurement of the serum level of HDAC1 in this skin disease.

PATIENTS AND METHODS

This was a case-control study comprised 45 patients suffering from AV. Additionally, 45 healthy subjects of matched age and sex were enrolled as a healthy control (HC) group. Patients receiving systemic and topical AV treatment in the last 2 months were ruled out.

METHODS

All cases were subjected to history taking, general and cutaneous examination and GAGS score calculation.

The GAGS divides the face (forehead (2), each cheek (2), nose (1), and chin (1)), chest, and back (3) into six regions, and the severity in each zone is then assessed on a scale of 0 to 4 (zero, no lesions; I, comedones; II, papules; III, pustules; and IV, nodules) [(8)]. The score for each area is measured by utilizing the formula: local score = factor x grade (zero-four). The global score is consisting of the total outcomes: 1-18-mild degree, 19-30-moderate degree, 31-38-severe degree, and above 39-acne with a very severe degree.

Laboratory work up:

A venous blood sample was obtained from each participant for measurement of serum level of human HDAC1using ELISA Kit (Cat. No E2041Hu, China).

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Specimen collection:

Three mL of venous blood was withdrawn from each participant under sterile situations; the blood samples were left until clotting, then sent for centrifugation at 3000 RPM for 15 min. The serum was kept frozen at -20°C till assay of HDAC1.

Ethical approval:

The study was approved by the Ethics Committee of the Faculty of Medicine at Mansoura University. A detailed description of the study's objectives was given to each adult participant or the caregiver of any child who took part in the study, before they completed an informed consent form. The Helsinki Declaration was adhered to at every stage of the investigation.

Statistical analysis:

Data were analyzed by SPSS program (IBM Corp. Released 2017, Version 25.0. Armonk, NY). Qualitative data were expressed as numbers and percentages. Quantitative data were expressed as means± standard deviation (SD), medians, and range. Kruskal-Wallis test: It is a non-parametric equivalent to ANOVA and used when ANOVA assumptions were violated to compare between more than two groups of skewed data. Significant results were judged at the (0.05) level.

RESULTS

Acne vulgaris group included 45 patients, 26 (71%) females and 19 (28.9%) males. Their age ranged from 16 to 25 years with a mean of 19.58 ± 2.46 years. Eleven (24.4%) patients had a positive family history **(Table 1)**.

Table (1): Comparison of demographic data, special habits, and anthropometric data between patients with AV and control group

	Acne vulgaris N = 45		**Control N = 45**		**Test (p)**
Age (years)					
Mean ± SD	19.58 ± 2.46		19.82 ± 2.87		X^2=0.434
Median (Range)	19.0 (16.0 – 25.0)		19.0 (16.0 – 27.0)		p-value=0.666
Sex	**№**	**%**	**№**	**%**	
Male	13	28.9%	9	20.0%	X^2=0.963
Female	32	71.1%	36	80.0%	p-value=0.327
Family history					
Absent	34	75.6	39	86.7%	X^2=1.813
Present	11	24.4	6	13.3%	p=0.178

SD: Standard deviation, Range: Min. – Max

The mean disease duration was 30.91± 3.94 months. The score of acne vulgaris ranged from 2 to 44, with a mean of 24.13 and the severity of AV was mostly moderate lesions in 27 (60%) patients **(Table 2).**

Table (2): Clinical characteristic of acne patients

	Acne vulgaris N = 45
Duration (months)	
Mean ± SD	30.91 ± 3.94
Median (Range)	24.0 (2.0 – 120.0)
Score	
Mean ± SD	24.13 ± 1.24
Median (Range)	23.0 (2.0 – 44.0)
Severity	
Mild	9 (20.0%)
Moderate	27 (60.0%)
Severe	6 (13.3%)
Very severe	3 (6.7%)

SD. Standard error, Range: Min. – Max

In the AV group, the mean serum HDAC1 level was 7.46±0.42 ng/mL (from 3.1 to 12.6 ng/ml). With regard to the controls, the mean serum level of HDAC1 was 1.34±0.07 (from 0.9 to 1.8 ng/ml). A significant difference between both groups was detected with regard to HDAC1 levels being significantly increased in the AV group (**Table 3**).

Table (3): Comparison of serum human HDAC1 level between patients with acne vulgaris and control group

	Acne vulgaris N = 45	**Control N = 45**	**Test (p)**
HDAC1 (ng/mL) Mean ± SD	7.46 ± 0.42	1.34 ± 0.07	U=4.50 p-value<0.001

SD. Standard error, Range: Min. – Max; U= Mann Whitney test.

HDAC1 showed a significant gradual increase in mild, moderate, severe, and very severe grades, respectively. HDAC1 displayed a significant positive correlation with the score of acne vulgaris. Greater HDAC1 was accompanied by the risk of AV susceptibility and acne vulgaris severity **(Table 4)**.

Table (4): Association between HDAC1 with severity of acne vulgaris.

	HDAC1 (ng/mL) Mean ± SD	**Test (p)**	**Pairwise Comparisons of Severity**		
Severity			Mild vs.	Mod vs.	Severe vs
Mild, n=9	4.01 ± 0.34	Kruskal-Wallis=33.896 p-value<0.001			
Moderate, n=27	7.31 ± 0.18		<0.001		
Severe, n=6	10.08 ± 0.29		<0.001	0.006	
Very severe, n=3	13.90 ± 2.45		<0.001	0.009	0.615

SD. Standard error, Range: Min. – Max

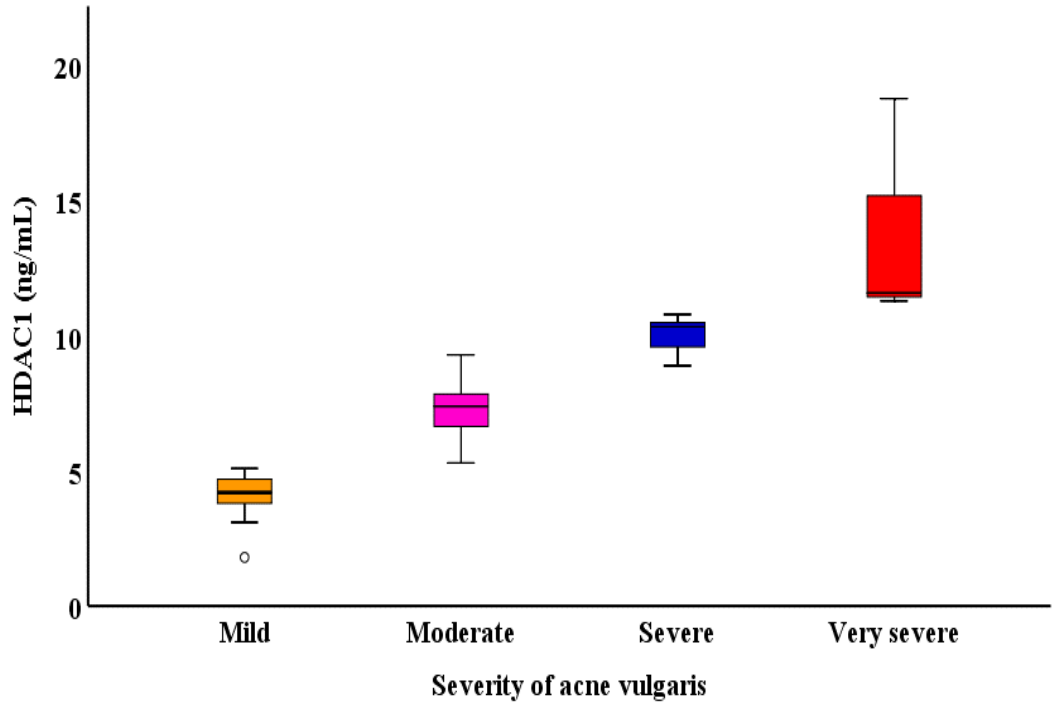

Figure (1): Boxplot for association between HDAC1 (ng/mL) with severity of acne vulgaris.

Table **(5)** shows no association between severity with age, sex, BMI, duration of the disease and family history.

Table (5): Association between severity with socio-demographic data, duration of disease and family history among patients with acne vulgaris

	Mild		**Moderate**		**Severe**		**Very severe**		**test**	**P**
	n=9		**n=27**		**n=6**		**n=3**			
Age (years)										
Mean ± SD.	20.2±3		19.6±2.5		18.5±1.5		19.7±2.1		F=0.573	0.636
Median (Range)	19(17-25)		19(16-25)		19(16-20)		19(18-22)			
Sex	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**		
Male, n=13	4	44.4%	5	18.5%	3	50.0%	1	33.3%	X^2=3.804	0.283
Female, n=32	5	55.6%	22	81.5%	3	50.0%	2	66.7%		
BMI (kg/m^2)										
Normal, n=34	7	77.8%	19	70.4%	5	83.3%	3	100.0	X^2=2.443	0.875
Overweight, n=9	2	22.2%	6	22.2%	1	16.7%	0	0.0%		
Obesity, n=2	0	0.0%	2	7.4%	0	0.0%	0	0.0%		
Mean ± SD	23.5±2.2		24±3.1		22.5±3.3		22±0.6		F=0.849	0.574
Median (Range)	23.7 (20.8-26.7)		23.5 (19.5-32.5)		22.6 (18.5-27)		21.7 (21.5-22.7)			
Duration of Disease										
Mean ± SD	35.1±14.3		32.5±4.5		25±4.5		16±4		H=2.086	0.555
Median (Range)	12(4-120)		36(2-96)		24(6-36)		12(12-24)			
Family history										
Absent (34)	4	44.4%	23	85.2%	5	83.3%	2	66.7%	X^2=6.397	0.094
Present (n=11)	5	55.6%	4	14.8%	1	16.7%	1	33.3%		

SE. Standard error, Range: Min. – Max; F, ANOVA; X^2, Chi square, H, Kruskal-Wallis test.

DISCUSSION

Acne vulgaris (AV) is an inflammatory skin lesion affecting mainly adolescents. It includes androgen-mediated increased sebum formation, abnormalities in keratinization, inflammation, and P. acnes-produced bacterial colonization **(3).** In addition, it is reported that genetic background also plays an essential role **(9).**

Histone acetylation has been considered a well-known modification. This process is adjusted by HATs and HDACs **(10).** Generally, acetylation is accompanied by transcriptional stimulation, while deacetylation has been demonstrated to be accompanied by gene repression. HDAC1 and HDAC2 were specially demonstrated to have a main function in ectodermal development (in the embryo) secondary to their existence in the epidermis and hair follicles **(11).**

Our study demonstrated that; the mean HDAC1 in cases with acne vulgaris was (7.46±0.42) versus (1.34±0.07) in the controls. Cases showed significantly greater HDAC1 levels compared to the controls (P<0.001). In agreement with our results, **Abdelkader *et al.*** **(12)** demonstrated a greater serum HDAC1 level in AV cases in comparison with HC. This also supports the outcomes of preceding research, which pointed to the alteration in the epigenetic processes in AV.

Propionibacterium acnes was demonstrated to suppress HDAC activity via the formation of short-chain fatty acids (SCFAs) causing increased cytokine formation secondary to the stimulation of Toll-like receptor II **(13)**. In addition, histone-4 (H4) is the main peptide formed by holocrine formation and it has antimicrobial effect against both staphylococcus aureus and P. acnes **(14)**. In addition, milk consumption (exacerbating factor of AV) could reduce DNA methyltransferase 1 expression and HDAC1 activity via micro-RNA signalling **(15)**.

This came in the same line with research that demonstrated that histone acetylation was elevated under inflammatory situations with SCFAs from Cutibacterium acnes. As a result, the process of histone acetylation modulation and the subsequent adjustment of extensive sebum formation and inflammatory responses may be an efficient treatment plan against AV **(13)**.

In addition, **Shin *et al.*** **(16)** displayed that HDAC1 may have an essential function in histone acetylation control and sebaceous lipogenesis in human and experimental studies. HDAC5 suppressed hepatic lipogenic gene expression by inhibiting the transcriptional activity of LXR **(17).** Of note, these researches recommend that individual kinds of HDACs control genes accompanied by lipid metabolism in various tissues.

In our study, HDAC1 showed a significant gradual increase in mild, moderate, severe, and very severe AV grades, respectively (p<0.001). HDAC1 displayed a significant positive correlation with the score of acne vulgaris (p<0.001). Greater HDAC1 was accompanied by the risk of AV susceptibility and acne vulgaris severity. **Abdelkader *et al.*** **(12)** reported no significant relationships determined between the serum HDAC1 level and age of cases with AV, severity and duration of AV (p>0.05). **Mohammadi *et al.*** **(18)** displayed that valproic acid suppresses HDAC in systemic lupus

erythematosus (SLE) cases, causing immunomodulatory actions on macrophages.

Souliotis *et al.* [19] recorded that vorinostat administration was associated with hyperacetylation of H4, chromatin decondensation, improvement of DNA repair efficacy, and reduction in apoptosis among cases with SLE. As a result, underexpression was recorded in genes included in DNA damage repair and signalling pathways, whereas overexpression was recorded in genes accompanied by apoptosis.

With regard to HDAC1 level, our study demonstrated that cases with AV were associated with a significant increase in HDAC1 level compared to HC. The actual mechanism of HDAC protein regulation is not totally identified. A lot of mechanisms were suggested, for example, protein complex formation, post-translational modifications (phosphorylation, and changes of gene expression) [20]. In addition, immunologic signaling pathways could adjust HDACs across various mechanisms, such as transcriptional regulation [21].

The actual explanation of the non-significant association between HDAC1 level and the evaluated parameters in cases with AV could be due to the fact that the complicated pathogenesis of AV and the pleiotropic feature of HDAC1 action.

CONCLUSION

The current study concluded that HDAC1 seems to be dysregulated in cases with AV. This could recommend a possible therapeutic opportunity for HDAC inhibitors in the context of AV management. Mush research is needed to explain this theory.

The multiplicity of the environmental factors that may interfere with HDAC level has been considered the main limitation.

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