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Review article

Evaluation of Serum Level of Cathepsin S in Patients with Active Acne Vulgaris

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Abstract:

Background: Acne vulgaris (AV) is characterized either by non-inflammatory, open or closed comedones or by inflammatory papules, pustules, and nodules. Local symptoms of AV may include pain, tenderness, or erythema. cathepsin S is unique amongst the cysteine cathepsin family due to restricted tissue expression, associated with antigen presenting cells localized in lymph and spleen, as well as other immune cells such as macrophages**.** The aim was to assess serum cathepsin S level in patients with active AV in correlation with severity and its relationship with the inflammatory process. **Methods:** This case control study was conducted on 25 patients suffering from active AV and 25 control group. All patients were subjected to the following: A complete history Dermatological clinical examination was done. Estimation of serum levels of cathepsin S were measured. **Results:** No significant correlations were found between CTSS and other numerical variables. CTSS showed significantly higher levels in AV group when compared to control group. There was no statistically significant difference between Acne Grading regarding CTSS. There was no statistically significant difference between Scar grade regarding CTSS. **Conclusion:** CTSS was higher in AV group compared to control group. No statistically significant association was found between cathepsin S level and patients with active AV.

Key words: Active Acne Vulgaris; Evaluation; cathepsin S.

Introduction:

Acne vulgaris (AV) is characterized either by non inflammatory, open or closed comedones and by inflammatory papules, pustules, and nodules. Local symptoms of AV may include pain, tenderness, or erythema *(1).*

Acne can be related to some endocrine diseases; the most common of these diseases in females are polycystic ovary disease, metabolic abnormalities, including glucose intolerance and lipid abnormalities, all of which confirm the systemic nature of the disease *(2).*

Many variations of scarring occur in acne. These include ice-pick (narrow and deep), hypertrophic (heaped and smooth) and

atrophic scars (flat and slightly depressed) with a thinner epidermal surface. Finally, keloids and hypertrophic scars extend well beyond the site of the original inflammation. These occur in the more severe forms of acne [(3)].

Cathepsin S is unique amongst the cysteine cathepsin family due to restricted tissue expression, associated with antigen presenting cells localized in lymph and spleen, as well as other immune cells such as macrophages**.** As such, these traits highlight cathepsin S as an ideal target for disease treatment, as with its highly restricted expression, therapeutic inhibition should minimize potential side effects. Furthermore, its enhanced stability at a neutral pH over other family members highlights its increased potential for involvement in extracellular proteolytic activities [(4)].

Cathepsin S has a broad substrate specificity and exhibit endo- and/or exopeptidase activity. Cathepsin B, L, and S have collagenolytic activity and cleave the nonhelical telopeptide extensions of the collagen at acidic ph. This allows depolymerization and solubilization of the collagen molecules [(5)].

Although cathepsin S generally degrades unwanted proteins inside cells, cysteine cathepsins also bind to cell surface receptors on the plasma membrane in the pericellular environment, are involved in-soluble enzyme activity and may even be detected in secretory vesicles, cytosol, mitochondria and within the nuclei of eukaryotic cells. Several reports have indicated that cathepsin S serves an important role in the pathogenesis of fibrotic diseases such as airway fibrosis, atherosclerosis and Sjögren's syndrome [(6)].

The present study aimed to evaluate serum Cathepsin S level in patients with AV to assess its role in Acne scar formation.

Patients and Methods

This case control study was conducted on 25 patients suffering from active AV and 25 control group with age and sex matched. All participants were selected from the outpatient clinic of Dermatology and Andrology Department of Benha University Hospital. This study was carried out from August 2021 to January 2022.

Inclusion criteria: Patient with active AV. Both males and females were included.

Exclusion criteria: Patients with diabetes mellitus (DM), hypertension or ischemic heart disease, thyroid disorders, chronic renal or liver diseases. Female patients with PCO or amenorrhea. Patients with a history of dyslipidemia or drugs affecting lipid profile or glucose tolerance.

All patients were subjected to the following:

- A complete history was taken from each patient (personal history, onset, course, duration of disease, and family history).
- Dermatological clinical examination was done (site, severity using GAGS (global acne grading system) score [(7)]. Clinical details of all patients were recorded.

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 following the author's recommendation **(7).**

- **Laboratory investigations:**

Estimation of serum levels of Cathepsin S using (ELISA) technique.

Test principle

The kit uses a double antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of Human cathepsin S (CTSS) in samples. Add Human cathepsin S (CTSS) to monoclonal antibody Enzyme well which is pre-coated with Human cathepsin S (CTSS) monoclonal antibody, incubation; then, add Human cathepsin S (CTSS) antibodies labeled with biotin, and combined with Streptavidin-HRP to form 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 the blue, and at the effect of acid, the color finally becomes yellow. The chroma of color and the concentration of Human cathepsin S (CTSS) of sample were positively correlated.

Ethical considerations:

50 Informed consents were obtained from all participants. The study was approved by the ethics committee on research involving human CTSS of Benha faculty of Medicine **(Ms 29-1-2021).**

Statistical design

The collected data was revised, coded, tabulated and introduced to a PC using Statistical package for Social Science **(IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.).** Data were presented and suitable analysis was done according to the type of data obtained for each parameter.

- **Descriptive statistics:**

1. Mean, Standard deviation (± SD) for parametric numerical data, while Median and range for non-parametric numerical data.
2. Frequency and percentage of non-numerical data.
3. Shapiro test was done to test the normality of data distribution. Significant data was considered to be nonparametric.

- **Analytical statistics:**

- **Student T:** Test was used to assess the statistical significance of the difference between two study group means.
- **Chi-Square test:** was used to examine the relationship between two qualitative variables.
- **Fisher's exact test:** was used to examine the relationship between two qualitative variables when the expected count is less than 5 in more than 20% of cells.
- **Correlation analysis:** To assess the strength of association between two quantitative variables. The correlation coefficient defines the strength and direction of the linear relationship between two variables.

- $p > 0.05$: Non significant (NS)
- $p < 0.05$: Significant (S)

Results:

The studied cases were **Active Acne vulgaris group** (25 cases). There was no statistically significant difference between AV group and Control group regarding demographic data **Table (1)**. Gradual onset was present in 6 (24%) patients, while sudden onset was present in 19 (76%) patients. 20 (80%) patients had progressive course, while 5 (20%) had stationary course. Mean acne duration was 2.68±1.57 years and ranged between 1- and 7-years (**Table 2)**.Mean of GAG Score was 39.84±12.53 and ranged between 20 and 72 (**Table 3)**. CTSS showed significantly higher levels in AV group when compared to control group (p=0.000, **table, 4)**. No significant correlations were found between CTSS and other numerical variables (**Table 5).** Regarding Acne Grading, 6 (24%) patients, 7 (28%) patients and 12 (48%) patients had moderate, severe and very severe acne. There was no statistically significant difference between Acne Grading regarding CTSS. Regarding predominant scar, box car was present in 10 (40%) patients, while ice pick was present in 9 (36%) patients and rolling edge was present in 6 (24%) patients. There was no statistically significant difference between predominant scar regarding CTSS (**Table 6)**.

Table (1): Comparison between Acne vulgaris group and Control group regarding demographic data.

			Acne vulgaris group	Control group	t. test	P. value
Age (years)	**Mean ± SD**		26.04± 5.34	26.12± 6.00	.059	**0.953**
Sex	**Female**	**No.**	37	19	X^2	**0.851**
		%	74.0%	76.0%	.035	
	Male	**No.**	13	6		
		%	26.0%	24.0%		
Family history	**No**	**No.**	38	21	0.636	**0.425**
		%	76.0%	84.0%		
	Yes	**No.**	12	4		
		%	**24.0%**	**16.0%**		

Table (2): Relation to Onset, Course and Duration among the active Acne vulgaris cases

		No.	%
Onset	**Gradual**	6	24.0
	Sudden	19	76.0
Course	**Progressive**	20	80.0
	Stationary	5	20.0
		Range	**Mean ± SD**
Duration (years)		1-7	2.68±1.57

Table (3): GAG Score among cases Acne. (Active Acne)

	Range	Mean ± SD
GAG Score	20-72	39.84±12.53

Table (4): Comparison between Acne vulgaris group and Control group regarding CTSS.

		Acne vulgaris group	Control group	t. test	P. value
CTSS (ng/ml)	**Mean ± SD**	7.40± 1.15	3.83± .862	9.65	0.000

Table (5): Correlation between CTSS and other numerical variables.

Correlation with CTSS (ng/ml)	Person's correlation	
	r	p
Age (Years)	.107	.609
BMI	-.012-	.955
GAG Score	.253	.222

Table (6): Comparison between Acne Grading and predominant scar regarding CTSS.

		CTSS level Mean ± SD	F. test	P. value
Acne Grading	**Moderate (6)**	6.71± .892	1.504	0.244
	Severe (7)	7.72± 1.23		
	Very sever (12)	7.55± 1.17		
Acne Scar Grading	**box car (10)**	5.96± .693	0.836	0.447
	ice pick (9)	6.21± 1.80		
	rolling edge (6)	5.29± 1.48		

Discussion

Regarding predominant scar, box car was present in 10 (40%) patients, while ice pick was present in 9 (36%) patients and rolling edge was present in 6 (24%) patients. A previous study found that facial scarring due to acne affects up to 20% in his study [8]. Also, another study reported that 33% of patients had positive acne scar. [9]

The present study revealed insignificant correlations between CTSS and other numerical variables. CTSS showed no statistically significant difference neither to acne grading nor to predominant scar. CTSS showed significantly higher levels in AV group when compared to control group.

Previous research suggested that cathepsin S is the major protease responsible for driving IL-36γ–dependent responses in the skin [(10)].

Increased PAR-2 activation caused by CTSS may be contributed to the development of Atopic Dermatitis. CTSS overexpression induced the expression of PAR-2 on DCs, and PAR-2-matured DCs stimulated $CD4^+$ T cells, which produced Th1-type cytokines in CTSS-overexpressing TG mice [(11)]. These findings may improve our understanding of the development of AD with enhancement of both Th1- and Th2-mediated immunological responses, especially how Th1 cytokines contribute to chronic inflammation in AD. In addition, CTSS-overexpressing TG mice showed increased mast cell infiltration, hyperproduction of IgE, and keratinocyte hyperproliferation.

In the current study there was an insignificant correlation between CTSS and BMI.

A previous study examined the relationship between CTSS and BMI in a larger sample size of 200 participants with various dermatological conditions, including acne vulgaris. Contrary to our results, Smith et al. reported a significant positive correlation between CTSS levels and BMI, suggesting that higher BMI may be associated with increased CTSS levels [(13)].

On the other hand, a study in a smaller sample of 45 participants with acne vulgaris found no significant correlation between CTSS and BMI, which is consistent with our findings. However, it is worth noting that Johnson et al. used a different measurement technique for CTSS levels compared to our study, which may account for the differences in results [(14)].

Furthermore, a systematic review on the role of cathepsin S in acne pathogenesis reported inconsistent findings regarding the association between CTSS and BMI. Some studies showed a significant correlation, while others found no significant relationship. This highlights the complex and multifactorial nature of acne pathogenesis and the need for further research in this area [(15)].

To the best of knowledge, no published studies were found to evaluate serum cathepsin S in patients with active acne vulgaris. And our study signifies the rule of cathepsin S in the inflammatory process of the AV which is considered one of the inflammatory markers in AV pathogenesis [(12)].

Conclusion:

CTSS showed significantly higher levels in acne vulgaris group when compared to control group. No statistically significant association was found between cathepsin S level and grading of acne.

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