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Serum Survivin Level as A Novel Biomarker in Acne Vulgaris Patients

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ABSTRACT

Background: Acne vulgaris is a common skin disease characterized by sebum overproduction, which is hormonally mediated, follicular hyper-keratinization and chronic inflammation of the pilosebaceous unit. Survivin is a member of inhibitors of the apoptosis (IAP) gene family. It is a 16.5 kDa protein that inhibits apoptosis and regulates cell division, proliferation, and survival.

Objective: To determine the survivin level in acne vulgaris patients and detect the relation of its levels with acne severity and presence of acne scarring.

Patients and Methods: Forty acne vulgaris patients were included in this case control study along with forty age and sex matched healthy controls. The patients were recruited from Dermatology, Venereology and Andrology Department, Faculty of Medicine, Zagazig University Hospitals in the period from October 2017 to May 2018.

Results: Results showed that there were statistical significant difference in survivin level among the three groups showing highest levels in scar group followed by active acne group then control group ($P < 0.001$). These findings support that there is a relationship between survivin and developing acne and acne scars. Moreover we found that there was statistical significant increase in survivin level among cases of active acne with progressive course compared to stationary cases ($P=0.001$). There was also statistical significant positive relation between survivin level and acne severity.

Conclusion: The findings of this study showed a significant association between survivin level and both active acne and acne scars, in addition to positive relation between survivin level and severity of acne. All these findings prove that survivin protein has a potential important role in acne pathogenesis and mechanism of acne scarring.

Keywords: surviving, acne vulgaris, acne severity.

INTRODUCTION

Acne vulgaris is a common skin disease characterized by sebum overproduction, which is hormonally mediated, follicular hyper-keratinization and chronic inflammation of the pilosebaceous unit [(1)]. Acne vulgaris is the eighth most prevalent disease worldwide. Almost every individual between 15 and 17 years of age is affected [(2)]. Acne may persist into adulthood in approximately 12%–14% of cases causing psychological and social implications due to disfigurement and permanent scarring [(3)]. Acne may be associated with lasting side effects, including facial scars, feelings of low self-esteem and withdrawal from society and depression [(4)].

Acne vulgaris is characterized by non-inflammatory open and closed comedo, and by inflammatory papules, pustules, nodules and sometimes purulent sacs. In some cases, acne is accompanied by scarring, a consequence of abnormal resolution or wound healing following the damage that occurs in the sebaceous follicle during acne inflammation. The scarring process can occur at any stage of acne [(5)]. It affects the area of the body, which contains hormonally sensitive sebaceous glands, including the face, neck, chest and upper arms [(6)]. Several independent, interacting factors contribute to acne pathogenesis, including increased sebum production, increased keratinization of the follicular epithelium, inflammation and overgrowth of normal skin microflora, particularly Gram-positive *Propionibacterium acnes* [(7)].

Survivin is a member of inhibitors of the apoptosis (IAP) gene family. It is a 16.5 kDa protein that inhibits apoptosis and regulates cell division, proliferation, and survival [(8)]. The expression of survivin is undetectable or is found at very low levels in normal tissues, whereas it is found at relatively higher levels in various malignant tissues, embryonic and fetal tissues, and uncommonly in normal adult tissues, including skin [(9)]. Interestingly, survivin was not previously evaluated, studied, described, or investigated in the fibrosis progression of the cutaneous tissues but its upregulation has already been described in certain liver diseases and during hepatic stellate cell activation. This proves to large extent the potential role of survivin in fibrogenesis process, most likely through regulation of apoptosis [(10)]. Survivin has been found to be increased in keratinocyte proliferative and inflammatory states, which are deeply involved in the pathogenesis of the acne lesions [(11)]. This may affect both the sebaceous gland (sebocyte survival) and the perifollicular dermal tissue (scar formation). Moreover, nuclear survivin

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expression has been observed in sebaceous hyperplasia and neoplasia [12].

The study aimed to determine the survivin level in acne vulgaris patients and to detect the relation of its levels with acne severity and presence of acne scarring.

PATIENTS AND METHODS

Patients:

Forty acne vulgaris patients were included in this case-control study along with forty age and sex matched healthy controls. The patients were recruited from Dermatology, Venereology and Andrology Department, Faculty of Medicine, Zagazig University Hospitals in the period from October 2017 to May 2018.

Ethical approval: Informed written consents were obtained from all participants after the approval of the Institutional Review Board of the Research Ethics (IRB), Faculty of Medicine, Zagazig University.

Study groups:

The participants were classified into two groups:

Group I (patients' group): Acne group contained 30 patients with active acne. Acne group was further subdivided into 3 subgroups according to global acne grading system into mild, moderate and severe. Each group contained 10 patients.

Group II: Scar group that contained 10 patients with acne scar.

Group II (control group) included forty healthy individuals of matched age and gender.

Inclusion Criteria:

- Acne vulgaris patients with either active lesions or acne scars.
- Sex: both sexes.

Exclusion criteria:

- Patients receiving any treatment for acne in the last 3 months or receiving oral isotretinoin in the previous 6 months.
- Patients with a history of chronic or acute hepatitis, liver cirrhosis, and benign or malignant tumors, as well as any other kind of cutaneous or fibrotic lesions which may be associated with elevated survivin level.

All participants were subjected to the following:

1. **Detailed medical history**:
   - Personal history including name, age, sex, marital status and educational grade.
   - Present history of acne vulgaris including onset, course, duration and hormonal state of females including menstrual cycle and hirsutism.
   - Family history of acne and acne scars.
   - History of previous treatment of acne.
2. **General examination**: to exclude any associated medical diseases.
3. **Detailed dermatological examination**:

a) **Complete skin examination:** including skin type, sites of acne, type of acne and type of scar (ice pick, boxcar, rolling).

b) **Acne severity is determined by Global Acne Grading System (GAGS):**

This system divides the face, chest and back into six areas (forehead, each cheek, nose, chin and chest and upper back) and assigns a factor to each area based on size as shown in table (1).

Table (1): Determination of acne severity according to GAGS

Location	Factor
Forehead	2
Right cheek	2
Left cheek	2
Nose	1
Chin	1
Chest and upper back	3

Each type of lesion is given a value depending on severity: no lesions = 0, comedo = 1, papules = 2, pustules = 3 and nodules = 4. The score for each area (Local score) is calculated using the formula: Local score = Factor × Grade (0-4). The global score is the sum of local scores, and acne severity that was graded using the global score. A score of 1-18 is considered mild, 19-30 moderate, 31-38, severe and > 39 very severe [13].

4. **Laboratory investigations :**
   - **Routine investigations:** as CBC
   - **Specific investigations:** detecting survivin level in serum using Enzyme Linked Immunosorbent assay (ELISA).

Statistical analysis

Data were checked, entered and analyzed using SPSS version 23 for data processing. The following statistical methods were used for analysis of results of the present study. Data were expressed as number and percentage for qualitative variables and mean ± standard deviation (SD) for quantitative one. Data were summarized using: The arithmetic mean ($\overline{X}$) and the standard deviation (SD).

ANOVA (F-test) test to calculate difference between quantitative variables in more than two groups. Chi- square (X^2) test to find the association between row and column variables. Correlation co-efficient rank test to rank different variables against each other in linear correlation, which was positive or negative. We consider (+) sign as indication for direct correlation i.e. increase frequency of independent lead to increase frequency of dependent & (-) sign as indication for inverse correlation i.e. increase frequency of independent lead to decrease frequency of dependent. In addition, we considered values near to 1 as strong correlation & values near to 0 as weak correlation. Level of significance: For all above-mentioned statistical tests, the threshold of significance was fixed at 5% level. P value of ≤ 0.05 indicates significant results.

RESULTS

Table (2): Demographic data of the three studied groups

Variable	Group I (Active acne) (n=30)		Group II (Acne scar) (n=10)		Group III (Control) (n=40)		F	p
Age (years): Mean ± SD	21.53 ± 2.57		23.00 ± 3.43		23.35 ± 3.85		2.56	0.08 NS
	No	**%**	**No**	**%**	**No**	**%**	χ^2	**p**
Sex:								
Female	13	43.3	7	70	20	50	2.10	0.60
Male	17	56.7	3	30	20	50		NS
Marital status:								
Married	6	20	5	50	10	25	3.55	0.17
Single	24	80	5	50	30	75		NS

SD: Standard deviation, F: ANOVA test, χ^2: Chi square test. NS: non-significant (P > 0.05)

This study included 80 participants, group I included 30 cases with active acne, their age ranged from 19-28 years with mean of 21.53 ± 2.57 years old, male to female ratio was 17:13 and 80% of cases were single. Group II included 10 cases with acne scar, their age ranged from 19-30 years with mean of 23.00 ± 3.43 years old, male to female ratio was 3:7 and 50% were married. Group III included 40 controls, their age ranged from 18-31 years with mean of 23.35 ± 3.85 years old, male to female was ratio1:1 and 75% were single. There were no statistical significant difference between the three studied groups concerning age, sex distribution or marital status as p value = 0.08, 0.60 and 0.17 respectively (Table 2).

Table (3): Clinical data among the two cases groups

Variable		Group I (Active acne) (n=30)		Group II (Scar) (n=10)		MW	p
Duration: (years)	Mean ± SD	1.09 ± 0.75		1.5 ± 0.52			0.07
	Median	1		1		1.87	NS
		No	**%**	**No**	**%**	χ^2	**p**
Course:	Stationary	5	16.7	---	---	---	---
	Progressive	25	83.3	---	---		
Onset:	Gradual	24	80	7	70	0.43	0.51
	Sudden	6	20	3	30		NS
Family history:	-ve	21	70	6	60	0.34	0.56
	+ve	9	30	4	40		NS
Previous ttt:	No	2	6.7	0	0		
	Yes	28	93.3	100	100	1.51	0.68
	Topical	14	46.7	4	40		NS
	Systemic	1	3.3	0	0		
	Topical & systemic	13	43.3	6	60		
Acne grade:	Mild	10	33.3				
	Moderate	10	33.3	---	--	---	--
	Sever	10	33.3				
Scar type:	Icepick			4	40		
	Rolling	---	--	3	30	---	--
	boxcar			3	30		

SD: Standard deviation. MW: Mann Whitney test. χ^2: Chi square test. NS: non-significant (P>0.05)

In group I (active acne), disease duration ranged from 2 months to 3 years with a mean of 1.09 ± 0.75 years, 83% of cases showed progressive course and 30% had positive family history. In group II (scar group), disease duration ranged from 1 – 2 years with a mean of 1.5 ± 0.52 years and 40% had positive family history. There was no statistical significant difference between the two groups in any of clinical data. Regarding scar type among scar group, 40% were icepick, 30% were rolling and 30% were boxcar (Table 3).

Table (4): Clinical history for the females of the acne group

Variable	N (n=13)	%
Menstrual history		
Regular	8	61.5
Irregular	5	38.5
Hirsutism		
No	12	92.3
Yes	1	7.7

Number of female patients in the acne group was 13 patients, 38.5% of them had menstrual irregularities and only one of them (7.7%) had hirsutism (Table 4).

Table (5): Comparison of survivin of the three studied groups

Variable	Group I (Active acne) (n=30)	Group II (Scar) (n=10)	Group III (Control) (n=40)	F	P	LSD
Survivin (pg/mL): Mean ± SD	123.1 ± 20.2	225.6 ± 60.7	53.8 ± 9.2	206.2	<0.001**	P1<0.001**[1] P2<0.001**[2] P3<0.001**[3]

SD: Standard deviation, F: ANOVA test, **: significant (P<0.05), LSD: Least significant difference
P1: Active acne versus scar, P2: Active acne versus control and P3: Scar versus control.

After measuring survivin levels in the serum using ELISA, group I levels range was 82.5 - 153.6 pg/mL with a mean of 123.1 ± 20.2 pg/mL. Group II levels range was 157.3 - 301.8 pg/mL with a mean of 225.6 ± 60.7 pg/mL and control group levels range was 41.5 - 73.8 pg/mL with a mean of 53.8 ± 9.2 pg/mL. There was a statistical significant increase in survivin level among scar group compared to active acne and control groups **(**P <0.001). There was also a statistical significant increase in active acne compared to control group as P < 0.001 (Table 5).

Table (6): Relation between survivin level and clinical and demographic data of group I

Variable		N	Survivin(pg/ml)			t	p
			Mean	Sd	Range		
Sex	Male	17	122.71	18.08	82.51 – 153.6	0.13	0.90
	Female	13	123.68	23.45	83.44 – 150.24		NS
Course:	Stationary	5	97.13	18.88	82.51 – 120.71	**3.82**	**0.001***
	Progressive	25	128.33	16.27	86.49 – 153.6		
Family histoy:	-ve	21	120.30	22.38	82.51 – 153.6	1.18	0.25
	+ve	9	129.73	12.52	113.21 -153.6		NS
Acne grade:	Mild	10	111.90	22.37	82.5 – 140.4	**F**	
	Moderate	10	120.92	16.98	86.49 – 140.87	**4.83**	**0.02***
	Sever	10	136.57	13.41	115.55 – 153.6		
Menstruation:	Regular	8	113.98	24.19	83.44 – 153.6	2.16	0.06
	Irregular	5	139.19	11.69	120.94 – 153.6		NS
Hirsutism	No	12	121.19	22.62	83.44 – 153.6	1.37	0.20
	Yes	1	153.6	-	153.6		NS

SD: Standard deviation. t: Independent t test. F: Anova test. NS: non-significant (P>0.05). *: Significant (P < 0.05)

This table showed that in group I, there was a statistical significant increase in survivin level among cases with progressive course compared to stationary cases (P=0.001). There was also statistical significant positive correlation between survivin level and acne severity (Table 6).

Table (7): Relation between survivin level and clinical and demographic data of group II

Variable		N	Survivin			t	p
			Mean	SD	Range		
Sex	Male	3	203.19	63.96	157.27 – 301.76	075	0.48
	Female	7	235.2	61.72	163.6 – 276.98		NS
Family histoy:	-ve	6	197.94	54.14	157.27 – 301.76	2.06	0.07
	+ve	4	267.09	48.52	195.82 – 298.84		NS
Scar type:	Icepick	4	211.54	59.79	163.6 – 298.84	**F**	
	Rolling	3	178.13	26.63	157.27 - 208.14	**5.8**	**0.03***
	boxcar	3	**291.81***	13.10	276.98 – 301.76		

SD: Standard deviation, t: Independent t test F: Anova test NS: non-significant (P>0.05) *: Significant (P<0.05)

On detecting relation between survivin level and both clinical and demographic data of group II, there was no statistical significant relation in all parameters except in scar type as there was statistical significant increase in survivin level among cases with boxcar scar compared to other types (Table 7).

Table (8): Correlation between survivin level and both age and disease duration among the two cases groups

Variable	Survivin			
	Group I (Active acne) (n=30)		Group II (Scar) (n=10)	
	r	P	r	P
Age	0.03	0.98 NS	0.25	0.49 NS
Duration	0.06	0.76 NS	0.47	0.17 NS

r: Pearsons correlation coefficient NS: Non significant (P > 0.05)

There was no statistical significant correlation between survivin and both of age and disease duration in cases groups (Table 8).

DISCUSSION

Forty acne vulgaris patients were included in this case-control study along with forty age and sex matched controls. Patients were divided to two groups; acne group that contain 30 patients with active acne who were subdivided according to GAGS into 3 subgroups (mild, moderate and severe), each group contained 10 patients and scar group that contained 10 patients with different types of acne scar.

There was no statistically significant difference between the three studied groups regarding demographic data. Clinical data among the two case groups also showed no statistical significant difference.

There was statistical significant difference in surviving serum level among the three groups showing highest levels in scar group followed by active acne group then control group (P < 0.001). This result is consistent with **Assaf *et al.*** [14]**.** Their study included 30 patients (15 with active acne and 15 with acne scar). They estimated the circulating levels and the expression pattern of survivin in the active acne and the acne scar groups in comparison with the healthy control group. They revealed that serum levels of survivin were markedly increased in the active acne ($p < 0.05$) and the acne scar groups ($p < 0.001$) in comparison with the healthy control group with an obvious increase in acne scar group compared to active acne group. Western blotting assessments showed that survivin expression was stronger in the acne scar group than in the active acne group and the control group. These results confirm our results. In addition, **El-Tahlawi *et al.*** [15] study, which included 30 acne patients (15 active acne and 15 post-acne scar) and 30 controls showed significantly high serum survivin levels between the control group and active acne and post-acne scar groups. These findings are in accordance with the present study. However, unlike our results, they found that patients with active acne attained a higher level of survivin than patients with acne scar. This difference may be due to different methodology as they used different grading system to classify disease severity (they used simple grading system whereas we used GAGS).

The exact mechanism of increasing survivin level in active acne cannot be determined, however many studies suggested this association [14]**.** Survivin had been found in keratinocytic proliferative and inflammatory states, which are deeply involved in the pathogenesis of the acne lesions**.** Abnormal apoptosis and enhanced sebocyte survival mediated by survivin might affect infundibular keratinocyte differentiation and alter sebum production leading to comedo formation and acne development. This evidences the role of survivin in the pathogenesis of acne [16]**.**

In post acne scar patients, increased survivin level was attributed to the potential role of survivin in fibrosis process as it has been shown that survivin has important role in fibrogenesis most likely through regulation of apoptosis**.** Elevation of survivin level in

post-acne scars may be owing to the inflammation and immune responses in acne, which increase oxygen consumption leading to localized tissue hypoxia**.** The continuous hypoxic environment can increase the expression of HIF 1α that can affect the expression of apoptosis molecules and inhibit apoptosis**.** Hypoxia-induced booster of survivin production has been associated with fibrotic remodeling and contributes to the up-regulation of survivin **(17).** In addition, increased survivin not only affects the sebaceous gland leading to acne formation but also affects the perifollicular dermal tissue leading to scar formation **(12).** For instance, increased survivin expression was found to contribute in fibroblast apoptosis resistance in idiopathic pulmonary fibrosis **(10)** and reversible liver fibrosis in which during the resolution phase of this fibrosis model, which is dependent on hepatic satellite cell apoptosis, there was a reduction of survivin expression to pre-fibrosis level **(18).** This demonstrates that survivin expression could have a mechanistic link with the pathogenesis of fibrosis in fibrotic disorders including acne-scarring process and thereby suppression of its expression could be a choice for the reversal of these lesions.

Regarding relation between survivin level and clinical and demographic data of active acne group, there were statistical significant increase in survivin level among cases with progressive course compared to stationary cases (P=0.001). There was also statistical significant positive correlation between survivin level and acne severity (P=0.02). These results are consistent with **El-Tahlawi *et al.* (15)** study as it showed significant increased levels of survivin with increased severity of acne and increased body mass index in patients with post-acne scar. The exact pathophysiology of the association between survivin level and severity of acne cannot be determined because studies exploring a relationship between serum survivin and active acne and post-acne scar are lacking. Only two prior studies have shown a link between them **El-Tahlawi *et al.* (15) and Assaf *et al.* (14).** However, some mechanisms could be suggested as increased survivin levels leads to increased abnormal apoptosis that affects sebocyte survival and amount of sebum production **(16).**

Regarding relation between survivin level and clinical and demographic data of acne scar group, there were no statistical significant relations in all parameters except in scar type as there was statistical significance increase in survivin level among cases with boxcar scar compared to other types. This may be due to the large surface area of boxcar scars in those patients. To our knowledge, our study is the first study to report this finding.

CONCLUSION

The findings of previous studies and the findings of this study support a significant association between survivin level and both active acne and acne scars in addition to positive relation between survivin level and severity of acne. All these findings prove that survivin protein has a potential important role in acne pathogenesis and mechanism of acne scarring.

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