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RESEARCH ARTICLE

Association between Survivin rs9904341 Polymorphisms and Susceptibility to Acne Vulgaris

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Abstract:

Background:

Acne vulgaris (AV), common dermatopathology, has a complex etiopathogenesis with a genetic background. The Survivin gene, which encodes an inhibitor of apoptosis protein, has been linked to some dermatologic disorders. The relationship between Survivin gene polymorphisms and AV has not yet been explored.

Objective:

To study the effect of survivin gene polymorphism rs9904341 and survivin serum concentration on the development of AV in Egyptian patients.

Methods:

Serum survivin was estimated using an enzyme-linked immunosorbent assay. Real-time quantitative PCR using allelic discrimination probes was conducted to investigate the rs9904341 polymorphism in the survivin gene in 118 AV patients and 120 healthy controls.

Results:

The serum survivin levels were significantly higher in AV patients than controls. Also, it was positively correlated with acne severity. The C allele was significantly more observed in acne patients compared to healthy controls. Patients with the C allele had 1.6 times higher odds of exhibiting acne than those with the G allele. The C/C genotype was significantly more observed in cases versus controls. Patients with the C/C genotype had 2.8 times higher odds of exhibiting acne as compared to those with the G/G genotype. However, no significant association was found between genotype distribution and grades of acne severity.

Conclusion:

Results showed significant associations between survivin gene rs9904341 genotypes and AV susceptibility, although it was not related to acne severity and could not be used as a marker of disease activity.

Abbreviations:

AV: Acne vulgaris, GAGS: Global Acne Rating System, SNPs: Single nucleotide polymorphisms.

Keywords: Acne vulgaris, Acne severity, Inhibitor of apoptosis proteins, Polymorphism, Promotor, Survivin.

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1. INTRODUCTION

Acne vulgaris (AV) is one of the chronic inflammatory skin diseases affecting all races all over the world. The symptoms of AV mostly seem in adolescence and teenagers, but they may also be observed in the forties and fifties of some people. Acne vulgaris is also one of the diseases that affect the patient's psychological and social status and quality of life, not only in teenagers but also in adults [1, 2]. Several pathological and physiological factors are responsible for the development of the disease, including androgen level, *Propionibacterium acnes* colonization, abnormal follicular keratinization, and

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extreme production of sebum. Currently, the involvement of inflammation in all kinds of AV has been demonstrated [3].

Human survivin is a 16.5 kDa protein coded by a Baculoviral IAP (Inhibitor of apoptosis proteins) repeat-containing 5 (*BIRC5*) gene in chromosome 17(17q25) at the telomeric position [4]. Survivin contributes to the apoptosis inhibitor (IAP) family, which is expressed in most human tumors but poorly found in normal adult tissue, including skin tissue. Survivin mediates abnormal apoptosis and enhances sebocyte survival, which can affect the differentiation of infundibular keratinocytes and modify the development of sebum, leading to the formation of comedo and acne [5, 6].

Single nucleotide polymorphisms (SNPs) located in the survivin gene promoter may be involved in changing the survivin expression level [7]. The SNP rs9904341 is located in regions encoding the cell cycle-dependent elements together with the cell cycle homology region repressor binding site [8], [9]. Rs9904341 (-31 G > C) polymorphism has been shown to have useful significance relative to other SNPs. In addition, other promoter polymorphisms of the survivin gene (-644T>C, -625G>C, and -241T>C; MAF>5%) were detected to be associated with rs99043411 [10]. Previous studies have shown that the C allele has substantially higher promoter activity than the G allele in this regard [9].

In this study, we aimed to elucidate the relationship between survivin gene polymorphism rs9904341 and the development of AV in Egyptian patients attending the Dermatology outpatient department in a university hospital. In addition, survivin serum levels of patients with AV were compared with those of a control population.

2. MATERIALS AND METHODS

2.1. Patients and Control Subjects

This was a case-control study with two groups of subjects. The first group consisted of AV patients. The second group consisted of acne-free, age- and sex-matched healthy volunteers. We included patients with no history of cancer or alcohol use and decided to participate in the research. Patients with systemic disorders (diabetes, hypothyroidism, or hyperthyroidism), pregnancy, malignancy, and systemic drug use were excluded.

All participants were subjected to full history taking, and the demographic and biochemical characteristics of individuals were recorded. The body mass index (BMI) was measured in units of kg/m^2 as the body mass divided by the square of the height of the body.

Acne severity was determined following the Global Acne Rating System (GAGS) into mild for patients with a score ≤ 18, moderate for patients with a score of 19 to 30, and severe for patients with a score ≥ 31 [11]. Informal written consent was obtained from all study participants before the study. The research has been approved by the Committee for Institutional Ethics (MS.19.12.960).

2.2. Sampling

All study subjects had 4 ml of peripheral venous blood drawn by venipuncture and divided into two aliquots. The first aliquot was used to obtain serum stored at -20 C for survivin concentration testing. The second aliquot was collected in EDTA vacutainers and stored at -20 °C to be used for DNA extraction.

2.3. Survivin rs9904341 Polymorphism Evaluation

The Quick-DNATM Miniprep Kit (ZymoResearch, USA, Cat. No. D3024) was used to isolate genomic DNA. The concentration and purity of the extracted DNA were assessed using the nanodrop 2000 Spectrophotometer (Thermo Scientific, USA). Agarose gel electrophoresis was used to test the integrity of the DNA.

2.4. Genotyping

Genotyping for rs9904341 in the survivin gene was conducted using a predesigned TaqMan SNP Genotyping Assay. The assay employed unique forward and reverse primers, in addition to TaqMan probes labeled with a fluorescent dye (either FAM or VIC). The survivin gene C/G (rs9904341) probes sequence was CCATTAACCGCCAGA TTTGAATCGC [C/G] GGACCCGTTGGCAGAGGTGGCG GCG. The mixture of reactions included 5µl of genomic DNA (200 ng/µl), 10µl of Taq-Man Master Mix (Thermo Fisher Scientific, catalog no. 4371353), 1µl of Taq-Man Genotyping Assay mix (Thermo Scientific, catalog number: 4351379), and H2O to achieve 20 µl final volume.

The PCR condition of the real-time allelic discrimination that was performed through the Step One Real-Time PCR system (Applied Biosystems, Foster City, USA) included: initially 30 seconds at 60 °C, followed by 10 minutes at 95 °C, and 40 cycles at 95 °C for 15 seconds then 60 °C for 1 minute) for annealing and extension. The integrity of the amplified DNA was tested using agarose gel electrophoresis. Genotyping was replicated on 10% of the samples that were randomly selected, and the results were 100% consistent.

2.5. Serum Survivin Level Estimation

After the collection of serum samples, serum survivin concentration is measured using a Human Surv (Survivin) ELISA Kit from Elabscience (USA), catalog No: E-EL-H1584, according to manufacturer's instructions. Sensitivity: 18.75 pg/ml. Detection Range: 31.25-2000 pg/ml.

2.6. Sample Size

The sample size was calculated by G*Power software (version 3.1.9.7). Following an earlier study by El-Mokadem *et al.* (2020) [12], the authors hypothesized that serum survivin levels would be statistically significantly higher between acne patients and control subjects with at least a medium effect size (d=0.5). Group sample sizes of 118 acne patients and 120 control subjects achieve 97% power to reject the null hypothesis of zero effect size when the population effect size is 0.50 and the significance level (α) is 0.050 using a two-sided two-sample equal-variance t-test.

As no previous similar research is available for the SNP study, our hypothesis was based on a pilot study performed in

our center on 20 acne patients and 20 healthy control subjects, which revealed a minor allele frequency of 30% in acne patients and 14.3% in control subjects. Accordingly, a sample size of 120 in each group (total 240) achieves 80% power with a 5% significance level. The sample size was calculated by Online Sample Size Estimator (OSSE): http://osse.bii.a-star .edu.sg/calculation1.php

2.7. Statistical Analysis

The **SNPStats** web tool was used to analyze the SNP. IBM-SPSS software (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.) was used to investigate the data. Qualitative data were presented as N (%) and compared by Chi-square or Fisher's exact test. Shapiro-Wilk's test was initially used to test for normality. The quantitative data used the median (25th -75th percentiles) and interquartile range (IQR) = 75th percentile minus 25th percentile. Quantitative data were compared by the Mann-Whitney U test for comparing two groups and Kruskal-Wallis H-test for comparing three groups. Correlation between two continuous variables or continuous and ordinal variables was done by Spearman's correlation, while a correlation between two ordinal variables was done by Kendall's tau-b correlation test. For each of the tests used, the statistically relevant findings were considered when the p-value ≤ 0.050.

3. RESULTS

The study included 118 patients with AV and 120 healthy individuals. The results showed statistically significantly higher proportions of survivin level (pg/ml) (P=<0.001), overweight/obese (P=0.001), and BMI value (kg/m^2) (P=0.001) in AV patients compared to healthy control subjects (Table **1**).

Table **2** shows a statistically significant higher C allele in patients with acne versus healthy control subjects (P=0.015). Participants carrying the C allele had 1.6 times higher odds than those carrying the G allele to exhibit acne. In addition, there was a statistically significant higher C/C genotype in AV cases compared to controls, with 2.8 times higher odds of exhibiting acne as compared to the G/G genotype.

For survivin rs9904341 polymorphism in AV patients, the recessive model is the best inheritance model (lowest p, AIC, and BIC values). Participants with the C/C genotype had 2.4 times higher odds of exhibiting acne than participants with G/G-G/C genotypes, regardless of age and sex. The SNP exact test for Hardy-Weinberg equilibrium (HWE) revealed a P-value= 1.0 for the control group, all alleles and genotype frequencies were in the range of HWE (Table **3**).

Table 1. Comparisons of clinical and laboratory parameters between the groups.

Parameter	Control	Acne	Statistic	P-value
N	**120**	**118**		
[a] Sex Female Male	91 (75.8%) 29 (24.2%)	100 (84.7%) 18 (15.3%)	2.982	0.084
[a] BMI category Ideal/underweight Overweight Obese	64 (53.3%) 30 (25%) 26 (21.7%)	35 (29.7%) 47 (39.8%) 36 (30.5%)	13.845	**0.001**
[b] Age (years)	22 (20-25)	22 (19-24)	-1.475	0.140
[b] BMI (kg/m^2)	24.9 (23.3-27.4)	26.8 (24.5-30.4)	-3.375	**0.001**
[b] Survivin level (pg./ml)	33.6 (20.4-84.6)	105.6 (57-135.6)	-7.788	**<0.001**

Data expression [test of significance]: [a] N (%) [Chi-Square test] and [b] Median (25th percentile – 75th percentile) [Mann-Whitney U-test].

Table 2. Allele and genotype frequency of survivin gene in acne cases vs. control.

Allele	Case	Control	χ^2	P1	COR (95% CI)	P2
N (participants)	**118**	**120**				
N (alleles)	**236**	**240**				
'G' allele	140 (59.3%)	168 (70%)	5.941	**0.015**	R 1.6 (1.1-2.3)	**0.015**
'C' allele	96 (40.7%)	72 (30%)				
Genotype	Case	Control	χ^2	P1	COR (95% CI)	P2
N (participants)	118	120				
G/G	46 (39%)	59 (49%)	6.463	**0.040**	R	R
G/C	48 (41%)	50 (42%)			1.2 (0.71-2.1)	0.461
C/C	24 (20%)	11 (9%)			2.8 (1.2-6.3)	**0.013**

COR: Crude odds ratio. R: Reference category. CI: Confidence interval. P1 value: Chi-Square test. P2 value: Binary logistic regression.

Table 3. SNP association with acne (n=238, adjusted by Age & Sex).

Model	Genotype	Case	Control	OR (95% CI)	P	AIC	BIC
N		**118**	**120**				
Codominant	G/G G/C C/C	46 (39%) 48 (41%) 24 (20%)	59 (49%) 50 (42%) 11 (9%)	R 1.2 (0.65-2.1) **2.6 (1.1-6.0)**	0.071	312.1	329.5
Dominant	G/G G/C-C/C	46 (39%) 72 (61%)	59 (49%) 61 (51%)	R 1.4 (0.83-2.4)	0.200	313.8	327.7
Recessive	G/G-G/C C/C	94 (80%) 24 (20%)	109 (91%) 11 (9%)	R **2.4 (1.1-5.4)**	**0.024**	310.4	324.2
Overdominant	G/G-C/C G/C	70 (59%) 48 (41%)	70 (58%) 50 (42%)	R 0.91 (0.53-1.6)	0.740	315.3	329.2
Log-additive	-	-	-	**1.5 (1.01-2.2)**	**0.044**	311.3	325.2

AIC: Akaiki Information Criterion. BIC: Bayesian Information Criterion.

Table 4. Comparisons between the three acne severity groups.

Parameter	Mild	Moderate	Severe	P-value
N	**41**	**53**	**24**	**-**
[a] Sex Female Male	35 (85.4%) A, B 6 (14.6%) A, B	50 (94.3%) B 3 (5.7%) B	15 (62.5%) A 9 (37.5%) A	**0.002**
[a] Genotype G/G G/C C/C	15 (36.6%) 16 (39%) 10 (24.4%)	22 (41.5%) 22 (41.5%) 9 (17%)	9 (37.5%) 10 (41.7%) 5 (20.8%)	0.935
[b]Age (years)	23 (20-27) A	22 (18-24) A, B	19.5 (19-21.8) B	**0.029**
[b]BMI	27.6 (25.9-30.5)	26.7 (25.7-30.4)	26.7 (23.3-31.3)	0.787
[b]Survivin concentration	80.4 (43.8-127) A	108.6 (66.6-141) A, B	126.6(89.7-168.3) B	**0.018**

Data expression [test of significance], [a] N (%) [Exact test], and [b] Median (25^{th} percentile – 75^{th} percentile) [Kruskal-Wallis H-test (KW)]. Pairwise comparisons for significant results by the KW test are presented in capital letters (similar letters = insignificant, different letters = significant).

Table 5. Correlation between acne severity and clinical and laboratory parameters.

Variable	Correlation coefficient	P-value
[a] Age (years)	-0.246	**0.007**
[a] BMI (kg/m^2)	-0.024	0.799
[b]BMI grades	0.018	0.825
[a] Survivin concentration	0.257	**0.005**

P-value: [a] Spearman's rho correlation test and [b]Kendall's tau-b correlation test.

As regards acne severity, there was a statistically significant difference in sex distribution, age, and survivin level between the three acne severity groups. Pairwise comparisons of moderate versus severe disease revealed a statistically significantly higher proportion of the female sex. Age was statistically significantly higher in mild versus severe diseases. Survivin level was statistically significantly higher in mild versus severe diseases (Table **4**).

Moreover, there was a statistically significant negative correlation of mild strength between acne severity and age. In contrast, there was a statistically significant positive correlation between acne severity and survivin level. However, there was no statistically significant correlation between acne severity and BMI (Table **5**).

4. DISCUSSION

This study was done to investigate the relationship between survivin gene polymorphism rs9904341, as well as survivin serum concentration and the development of AV in Egyptian patients. To the best of our knowledge, this is the first study that addresses the relationship between survivin gene polymorphism and acne susceptibility. This might help better understand the factors affecting individual susceptibility to develop AV based on their genetic background.

Our findings demonstrate that the frequency rates of C variant alleles and C/C genotype were significantly higher in AV patients than in those of the control group. Compared to individuals with the variant homozygous genotype C/C, those with G/C, G/G, and wild type (G/C + G/G) showed a decreased risk of developing AV. In contrast with those carrying variant allele C, individuals carrying allele G have demonstrated a lower risk of developing AV.

Our results showed no significant relationship between genotype distribution and different grades of acne severity. Subsequently, the rs9904341 polymorphism of survivin promoter could not be used as a marker for acne severity. However, the AV group had significantly higher serum survivin levels than the control group. Also, it was positively correlated with acne severity, where serum survivin levels were significantly higher in severe versus mild disease. This result is supported by previous similar findings by Assaf *et al.* [13], El-Tahlawi *et al.* [14], and Hanum *et al.* [15], who showed increased survivin levels with an increase in the severity of AV and the development of acne scars.

The relationship between acne severity and survivin level could be explained by the fact that an increase in survivin levels can lead to abnormal apoptosis in keratinocytes and sebocytes, which will increase sebocyte survival and increase the amount of sebum production, causing acne to be more severe [14]. This study could benefit future studies to target survivin for acne treatment, especially the severe form of acne that can affect the patient's quality of life.

This study has some limitations in that it is monocentric in nature. Additional studies can be conducted to compare survivin SNP with the previously studied biomarkers related to acne severity in terms of specificity, sensitivity, ease of identification or prognosis.

CONCLUSION

In conclusion, in the studied group, the rs9904341 polymorphism of survivin promoter (C/C genotype) was associated with AV susceptibility. In addition, a positive relation between survivin level and acne severity was recorded. This observation makes survivin a suitable target for therapy. Survivin gene rs9904341 polymorphism was not related to acne severity and could not be used as a marker of disease activity.

LIST OF ABBREVIATIONS

AV	=	Acne Vulgaris
GAGS	=	Global Acne Rating System
SNPs	=	Single Nucleotide Polymorphisms

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The research has been certified by the Committee for Institutional Ethics (MS.19.12.960).

HUMAN AND ANIMAL RIGHTS

No animals were used in this research. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or research committee and with the 1975 Declaration of Helsinki, as revised in 2013.

CONSENT FOR PUBLICATION

Informed consent was obtained from all participants of this study.

STANDARDS OF REPORTING

STROBE guidelines were followed.

AVAILABILITY OF DATA AND MATERIALS

The data will be available from the corresponding authors [E.E.] upon request.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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