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Serum Level of Survivin in Patients with Alopecia Areata and its Association with Disease Severity and Progression

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Abstract

Background: **Alopecia areata (AA) is an acquired cytotoxic T-cell immune-mediated hair loss disorder. The autoimmune attack triggers apoptosis with rapid progression of hair follicles (HFs) from the anagen to catagen and telogen phases of the hair cycle. Survivin is an inhibitor of apoptosis protein responsible for cell cycle regulation and apoptosis inhibition. Its expression has been determined in proliferating anagen HF cells, whereas its expression prominently decreases during the catagen phase.** ***Aim:*** **This study aimed to evaluate serum survivin level in AA patients and its relationship with AA severity and progression.** ***Subjects and Methods:*** **The study included 38 AA patients and 38 healthy controls. Alopecia areata severity was assessed by the severity of the Alopecia Tool score. Serum survivin level was measured by enzyme-linked immune-sorbent assay.** ***Results:*** **Serum Survivin level was significantly lower in AA patients than in controls (*p*= 0.009). Moreover, the level was significantly lower in patients with progressive disease than in those with stable disease (*p*<0.001). A significant negative correlation was found between survivin serum level and AA severity (*p* < 0.001) and duration (p=0.003).** ***Conclusion:*** **Survivin serum level was decreased in AA patients. Thus, survivin could have a potential role in AA immune pathogenesis and its serum level might be a predictor for AA severity and progression.**

Keywords: Hair follicles, Alopecia areata, Survivin, autoimmunity

Introduction

Alopecia areata (AA) is a commonly acquired immune-mediated non-cicatricial loss of hair. A cytotoxic T-cell autoimmune attack against the anagen hair follicle (HF) is implicated in its development[(1)]. This attack triggers a rapid progression of HFs from anagen into catagen and telogen phases of the hair cycle. Less severely affected follicles continue in anagen but develop dystrophic hair shafts with increased shaft fragility that ultimately proceeds to telogen[(2)]. This HF regression is associated with a series of signaling pathways that trigger apoptosis in HF cells[(3)]. Survivin is one of the inhibitors of apoptosis proteins that has a pivotal function in mitosis regulation and apoptosis inhibition[(4)]. Cytoplasmic survivin creates a complex with the x-linked

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inhibitor of apoptosis proteins and the complex binds to caspase-3 to repress its apoptotic function. Nuclear survivin serves as a transcription factor during phase G2/M of mitosis, promoting cell growth[5]. Survivin has been identified in proliferating anagen HFs matrix cells and outer root sheath keratinocytes, while its expression decreases during the HF regression in the catagen phase[6]. This study was designed to evaluate the possible role of survivin, as an antiapoptotic protein, in the pathogenesis of AA.

Patients and Methods

The included patients were recruited from the dermatology clinic of the Dermatology Department. Written consents were signed by subjects and approvals of the local ethics committee were obtained. 38 patients with different types of AA and 38 age and sex-matched healthy volunteers were enrolled in this case-controlled study. Alopecia areata was considered acute if presenting for less than 6 months and chronic if presenting for more than 6 months. Subjects aged <16 years at the time of onset were considered to have juvenile onset AA and those older than 16 years at the time of disease onset were considered suffering of mature onset AA. Males and females aged between 16 and 60 years complaining of different patterns and severity grades of AA were included in the study. Those complaining of autoimmune diseases, associated skin inflammatory conditions, and receiving immunosuppressive medications were excluded from the study. Any subject who received topical, intralesional, or systemic treatments for AA should have maintained 3 months wash-off period prior to inclusion. Subjects suffering from active acne, acne scars, melanoma, or non-melanoma skin cancers (NMSC) were similarly excluded from the study. All patients were subjected to history taking, general examination as well as assessment of the extent and severity of their alopecia patches using the Severity of Alopecia Tool score (SALT score) which determines the percentage of hair loss in the scalp[7]. Based on the percentage of hair loss, AA was graded into mild AA; with <25% area of the scalp being involved, moderate AA; with 25% up to <50% area of the scalp being involved and severe AA; with 50% to 100% area of the scalp being involved (including Alopecia totalis, Alopecia universalis, and ophiasis). Next, the body was carefully examined to detect any alopecia patches in any hairy area, and a nail examination was carried out to detect any nail involvement. Finally, a trichoscopy was performed by a trained dermatologist to confirm the diagnosis of alopecia areata. A 5 ml blood sample was collected from the peripheral veins of each participant in this study and serum survivin level was determined by Enzyme-Linked Immune-Sorbent Assay (ELISA).

Statistical Analysis

SPSS v.25 was used for statistical analysis. ANOVA testing of variance was used to compare both groups. Chi-square (χ^2) test, T-test, and Pearson correlation analysis were calculated and a $P < 0.05$ was considered statistically significant.

Results

The study included 38 AA patients 25 (65.8%) females and 13 (34.2%) males; their demographic criteria are shown in Table (1), their ages ranged from 16 to 53 years with a mean of 24.42 ± 10.34 years, whereas that of HCs ranged from 17 to 54 years, with a mean of 26.76 ± 10.75 years. Regarding AA severity, 27 (71.1%) patients were S1 (mild alopecia), seven (23.7%) patients were S2 (moderate alopecia), and two (5.3%) patients were S3 (severe alopecia). Their

clinical characteristics are shown in Table (1). In the study group, serum survivin levels ranged from 60.0 to 230.0 pg/ml, with a mean of 105.76 ± 29.07 pg/ml. In HC, the level ranged from 55.0 to 400.0 pg/ml, and the mean was 149.55±86.87 pg/ml (Table 2), this difference between both groups was statistically significant (*p*= 0.009) (Figure 1).

Table 1: Demographic data and clinical characteristics of AA patients (n=38)

	No.	%
Sex		
Male	13	34.2
Female	25	65.8
Age of patients (years)		
Min. – Max.	16.0 – 53.0	
Mean± SD.	24.42 ± 10.34	
Median	20.0	
Age of disease onset (years)		
Min. – Max.	0.25 – 16.0	
Mean ± SD.	1.36 ± 2.04	
Median	2.0	
Duration of the biopsied lesion (months)		
Min. – Max.	0.25 – 36.0	
Mean ± SD.	6.12 ± 9.07	
Median	2.0	
Disease course		
Progressive	14	36.8
Stationary	24	63.2
Nail abnormality	2	5.3
Site of AA lesion		
Scalp and body sites	4	10.5
Scalp only	34	89.5
Family history (AA, atopy, Autoimmune D.)	9	23.7
Previous AA attacks	4	10.5
Being atopic	10	26.3
Clinical pattern		
Patchy	33	86.8
Single	19	50.0
Multiple	14	36.8
Ophiasis	5	13.2
Severity	**No.**	%
Mild (S1) (< 25%)	27	71.1
Moderate (S2) (25% - <50%)	9	23.7
Severe (S3) (50-74%)	2	5.3
SALT score		
Min. – Max.	2.0 – 55.0	
Mean ± SD.	15.26 ± 14.96	
Median	8.0	

Min: minimum; Max: maximum; SD: standard deviation; AA: alopecia areata; D: diseases; SALT: Severity of Alopecia Tool.

The mean serum survivin level in patients presenting with single patch AA was 120±31.45pg/ml, compared to 86.07 ± 13.33pg/ml in patients with multiple patches AA and 107 ± 21.68pg/ml in patients with ophiasis. The difference between survivin levels among different patterns of AA was statistically significant (p<0.001). In addition, the level was significantly lower in patients with progressive disease (90.36 ±16.58 pg/ml) than in those with stable disease (114.8 ±31.22 pg/ml) (p= 0.002). Furthermore, patients with severe AA (S3) had a significantly lower serum survivin level (80 ±7.07 pg/ml) compared to moderate cases (S2) (83.33 ±10.9pg/ml) and mild cases (S1) (115.2 ±29.1pg/ml) (*p*= 0.001) (Table 3). A significant negative correlation was found between serum survivin and AA severity (p< 0.001) (Fig. 2) and duration (*p*= 0.003) (Fig. 3). However, no association was established between serum survivin levels and other clinical characteristics (patients' gender, age, age of disease onset, positive family history, previous AA attacks, and atopy (Table 3).

Table 2: Survivin serum level among AA patients compared with healthy controls

Serum survivin (pg/ml)	**Controls** (n = 38)	**AA patients** (n = 38)	U	P
Min. – Max.	55.0 – 400.0	60.0 – 230.0	469.50*	0.009*
Mean ± SD.	149.55 ± 86.87	105.79 ± 29.07		
Median	127.50	95.0		

U: Mann Whitney test; significant at p < 0.05. AA: alopecia areata.

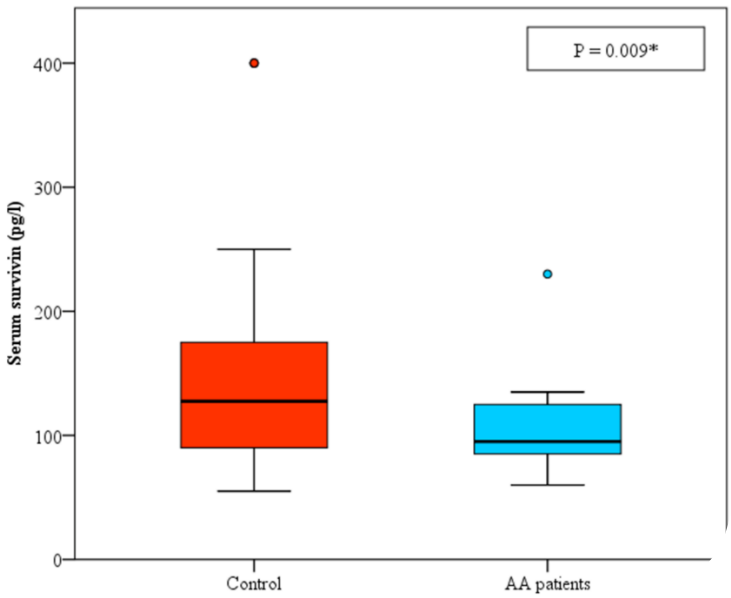

Figure 1: Serum level of survivin among AA patients (n=38) compared with healthy controls

P-value is for Mann Whitney test. Serum level of survivin in AA patients (mean= 105.79 ± 29.07 pg/ml, median= 95.0 pg/ml); and in controls (149.55 ± 86.87 pg/ml, median= 127.50 pg/ml).

*****: Statistically significant at p< 0.05. AA: alopecia areata.

Discussion

The pathogenesis of AA entails apoptotic regression of anagen HFs triggered by cytotoxic T-cells[8]. Survivin is an antiapoptotic protein with significantly decreased expression during the progression from anagen to catagen phase of the hair cycle. Survivin has a dual function that may be implicated in the regulation of the precise proliferation–apoptosis balance that controls the HF cycle[6]. The role of survivin in AA pathogenesis has been poorly investigated. To date, this is the first work done to assess serum survivin levels in AA patients, up to the best of our knowledge. In this study, there were lower serum survivin levels in AA patients than in HC. This low level might have a role in triggering apoptotic activity in HFs encountered in AA.

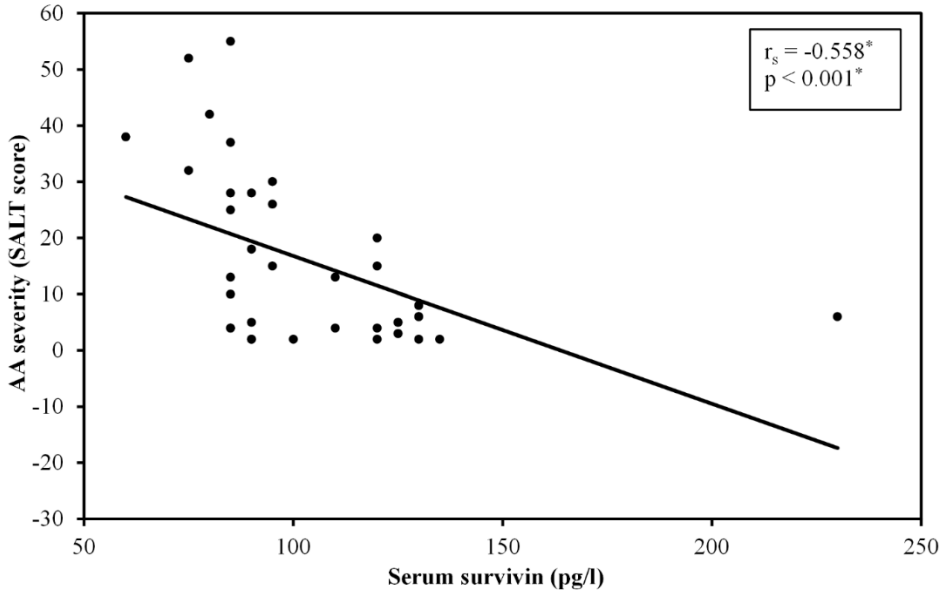

Figure 2: Correlation between survivin serum level and SALT score of AA patients
*r: Spearman coefficient. *: Statistically significant at p < 0.05. SALT: Severity of Alopecia Tool. AA: alopecia areata.*

Interestingly, Simonetti et al.[9] showed that survivin immune positivity was faint in HFs, keratinocytes, and endothelial cells of AA lesions. After topical diphencyprone application, this immune positivity was markedly increased in the endothelial cells but slightly decreased in the HFs. They suggested that survivin might have a role in maintaining the survival of endothelial cells during the angiogenesis crucial for hair regrowth in AA lesions. In contrast to these results, Marie et al.[10] reported higher survivin gene expression in AA lesions compared with non-lesional and control biopsies and postulated that survivin might have a role in maintaining the survival of auto-reactive T cells in AA lesions. The discrepancy between these data may suggest a dual function of survivin in AA pathogenesis. Survivin gene expression may have been significantly elevated locally in AA lesions, and the resultant protein may have been consumed in promoting the survival of auto-reactive T cell infiltrates, leading to a reduction in its serum level, which may have triggered the apoptotic cascade in anagen HFs, and their regression into catagen then telogen phases. This postulation was confirmed by the markedly increased survivin expression in endothelial cells and its decreased expression in HFs of AA lesions following DPCP treatment[9]. Several

studies investigated the role of survivin in autoimmune and skin inflammatory diseases. In agreement with this study, Ebrahimian *et al.*[11] found that survivin serum level has been decreased in systemic lupus erythematosus patients and postulated that these low levels might have a pivotal role in the acceleration of the apoptotic cascade encountered in this disease. In contrast to our results, Marie et al[12] reported significantly higher survivin serum levels in vitiligo patients compared with HCs and stated that survivin helps maintain the survival of auto-reactive T cells.

Table 3: Relation between survivin serum level and the different characteristics of AA patients (n= 38)

	N	**Serum survivin** (pg/ml)			Test of Sig.	P
		Min. – Max.	Mean ± SD.	Median		
Gender						
Male	24	60 – 230	106.3 ± 32.81	95.0	U=166.0	0.964
Female	14	75 – 135	105.0 ± 22.36	107.5		
Age at AA onset (years)						
Adolescent (<18)	13	75.0 – 135	107.31 ± 21.47	120	H=0.544	0.762
Young adults (18-35)	21	60 – 230	106.4 ± 34.65	95		
Middle-aged adults (36-55)	4	85 – 130	97.50 ± 21.79	87.50		
Previous attacks						
No	34	60 – 135	103.8 ± 20.89	97.5	U=64.50	0.873
Yes	4	75 – 230	122.5 ± 72.17	92.5		
Clinical pattern						
Single patch	19	85 – 230	120 ± 31.45	120	H=15.290*	<0.001*
Multiple patches	14	60 – 120	86.07 ± 13.33	85		
Ophiasis	5	85 – 130	107 ± 21.68	100		
Duration (months)						
≤10	32	60 – 230	109.06 ± 30.5	110	U=49.0	0.003*
>10	6	85 – 100	88.33 ± 6.06	85		
Severity (SALT score)						
Mild (S1)	22	85 – 230	117.1 ± 30.65	120	H=11.457*	0.001*
Moderate(S2)	9	60 – 95	83.33 ± 10.90	85		
Severe (S3)	7	75 – 130	99.29 ± 22.25	90		
Family history						
Negative	29	60 – 230	105 ± 32.15	90	U=95.50	0.234
Positive	9	90 – 130	108.3 ± 16.77	110		
Course						
Stationary	24	75 – 230	114.8 ± 31.22	120	U= 67.50*	0.002*
Progressive	14	60 – 120	90.36 ± 16.58	85		
Atopy						
Negative	28	60 – 230	106.6 ± 32.43	97.50	U=129.0	0.732
Positive	10	85 – 135	103.5 ± 17.65	95.0		

U: Mann Whitney test; H: Kruskal Wallis test. Significant at p < 0.05. AA: alopecia areata; SALT: Severity of Alopecia Tool.

Likewise, a number of psoriasiform conditions [psoriasis[13,14], lichen simplex chronicus, and thickened plaques[15]] had been linked to increased survivin expression. Even though the exact function of survivin in psoriasis remains not fully clear, it was linked to increased keratinocyte survival as well as increased angiogenesis[14]. Serum and tissue expression of survivin were reported to be significantly high among patients suffering from active acne and/or acne scars. The authors postulated that survivin has a pivotal role in acne development and scar formation through stimulated angiogenesis as well as prolonged sebocyte survival[16]. The current study demonstrated that survivin serum level was significantly reduced in AA patients with multiple patches than in patients suffering from ophiasis and/or single-patch AA. Furthermore, the levels were also significantly reduced in patients with a progressive disease course than in those with a stable disease course. There has been a significant negative correlation between survivin serum levels and AA severity and duration. These findings are consistent with the previously reported role of survivin in the regulation of apoptosis implicated in AA lesion development and might propose survivin serum level as a predictor of AA severity and progression. On the contrary, El-Tahlawi *et al.*[16] revealed a positive correlation between the severity of acne vulgaris and survivin serum levels, and Marie *et al.*[12] showed a significant positive correlation between serum level of survivin and vitiligo severity. Limitations to the study included the limited number of patients participating in the study, so larger sample size studies are encouraged.

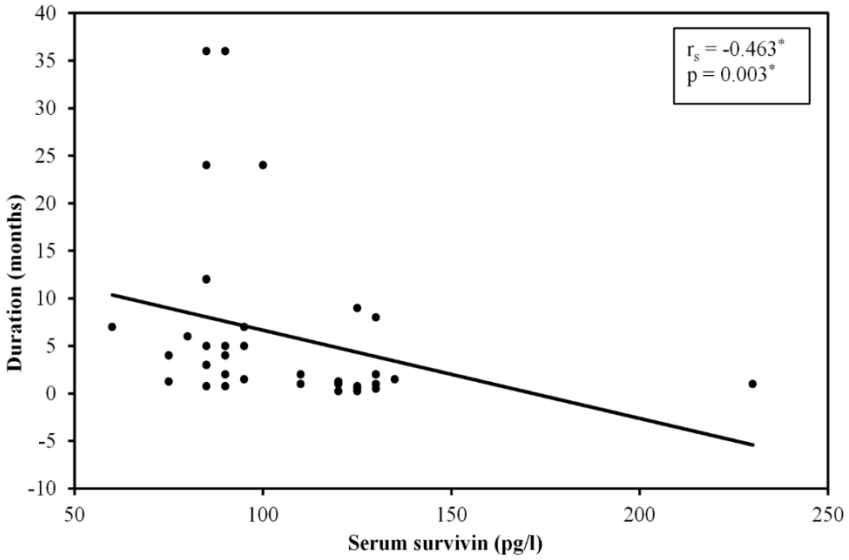

Figure 3: Correlation between survivin serum level and disease duration (months) in AA patients
r: Spearman coefficient. ***:** Statistically significant at $p < 0.05$. AA: alopecia areata.

Conclusion

Survivin serum level was decreased in AA patients, and this decreased level was associated with AA severity and progression. Thus, survivin could be of a potential role in AA immunopathogenesis and its serum level might be a predictor for AA severity and progression. Further identification of the molecular roles of survivin and the

regulation of its expression in HFs can shed new light on nontraditional strategies for the medical treatment of AA.

Availability of data and materials
All data and materials related to the present work have been included in this article.

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Conflicts of Interest
The authors have no conflict of interest to declare.

Ethical considerations
The study had the approval of the local Institutional Review Board and the Research Ethical Committee, Faculty of Medicine, Suez Canal University. All participants filled out a written informed consent to participate in the study.

Authorship
All authors had made substantial contributions to all of the following: (1) the conception and design of the study, (2) the acquisition of data, data analysis, and interpretation, (3) drafting the article and revising it critically, (4) final approval of the submitted version, (5) Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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