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Evaluation of Serum Calprotectin as a Marker of Activity in Patients with Alopecia Areata

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ABSTRACT

Background: The course of alopecia areata (AA) is unpredictable with periods of activity and periods of remission. Active alopecia areata usually require more aggressive treatment to stop disease progression. Calprotectin is a promising marker of inflammation that could predict a serological relapse before a clinical one in several inflammatory diseases.

Objective: To estimate calprotectin level in the serum of alopecia areata patients and detection of the relation between serum calprotectin and activity in alopecia areata.

Subjects and methods: Sixty subjects were included in this study, 20 patients with active AA, 20 patients with stable AA and 20 controls. Serum calprotectin levels were measured in the 3 groups by using ELISA.

Results: Serum calprotectin level was higher in AA patients than in controls but with no statistically significant difference. Active alopecia areata patients had higher serum calprotectin than those with stable alopecia areata but the difference wasn't statistically significant.

Conclusion: Although calprotectin is a marker of inflammation in many different inflammatory disorders, its serum level was not a predictor for activity in AA patients.

Keywords: AA, Serum calprotectin, Disease activity.

INTRODUCTION

Alopecia areata (AA) is a prevalent non-cicatracial hair loss disorder that is immune-mediated and persistent. AA prevalence is about 2% of the general population with no gender or racial differences. AA may start at any age but its onset is common in the twenties or thirties [1].

Patients of alopecia areata present with sudden appearance of patches that are completely devoid of hairs [2]. Usually, AA is diagnosed by a clinician. Alopecia totalis, which is the total loss of scalp hair, and alopecia universalis, which is the total loss of body hair, are the two clinical presentations of AA [3].

The clinical characteristics of AA include intact ostia of hair follicles, positive hair pull test, complete absence of terminal hairs in the patch, in addition to absence of active inflammation signs of hair follicles e.g. perifollicular erythema, scaling or pustules [4]. The course of AA is unpredictable with periods of remission and relapse. AA course may be persistent, especially in severe hair loss [3]. AA may affect patients' quality of life significantly and may lead to anxiety or depression [5].

Clinically, the hair pull test and the search for exclamation hairs around the AA patch's boundaries can be used to measure the activity of AA [6]. Hairs with an exclamation point are thought to be pathognomonic for AA [7]. Dermoscopy can confirm the diagnosis of AA [8, 9], and it can help in determination of hair loss activity by coexistence of several trichoscopic findings like yellow dots, black dots, exclamation marks [10]. AA may be accompanied by nail changes or other autoimmune disorders like systemic lupus erythematosus (SLE) [11]. The etiopathogenesis of AA may involve a variety of factors, including genetic susceptibility, autoimmunity, environmental factors, and emotional factors [12-14]. The primary prerequisite for the emergence of AA is the disintegration of the hair follicle's immune privilege [15]. It is thought that AA is a T-cell-mediated hair loss condition that can lead to systemic inflammation. AA may be a systemic condition, according to certain research, which supports the need for immune-based systemic therapy [16].

S100A8 and S100A9 proteins are the two components that form a heterodimer complex, called calprotectin, they are mainly secreted by stimulated monocytes and neutrophils in blood, body fluids and inflamed tissues. Calprotectin has immunomodulatory, antimicrobial, and antiproliferative functions [17-19]. Calprotectin is suggested to be a sensitive and promising biomarker of inflammation indicating inflammatory activity even in subclinical phases [20]. Fecal calprotectin can be used to identify inflammatory bowel disease (IBD) activity, forecast relapse, and track therapeutic response. It is a valid diagnostic tool for distinguishing IBD from GIT functioning disorders [21].

Many conditions have been related to calprotectin levels, such as SLE, polymyalgia rheumatica, ankylosing spondylitis, rheumatoid arthritis, systemic vasculitis, Still disease, and Sjogren's syndrome [22]. Serum and synovial calprotectin levels have been linked to acute phase reactants and disease activity [23]. Several investigations have found a substantial link between serum calprotectin and inflammatory skin illnesses such as psoriasis, hidradenitis suppurativa, acne vulgaris, and atopic dermatitis [24].

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PATIENTS AND METHODS

The study was designed as a prospective, age matched case-control research. Sixty participants were included in this study, 20 patients in active stage of alopecia areata, 20 patients in inactive stage of alopecia areata and 20 healthy controls.

Every patient underwent a comprehensive process of obtaining their complete medical history and conducting a meticulous general examination. Local dermatological and dermoscopic examination were performed. Hair pull test was performed in all patients. AA was considered in active stage if there were exclamation hairs clinically, positive hair pull test and activity was confirmed by detection of dermoscopic signs of activity like (yellow dots, black dots, exclamation marks, tapering hairs and broken hairs).

Following consent, serum samples from both volunteers and patients were taken. 4 cubic centimeters of venous blood were withdrawn and centrifuged for 5 minutes. Before analysis, serum samples were kept in storage at -20 °C. Serum calprotectin levels were measured in the 3 groups by using the ELISA (Bühlmann Laboratories AG, Switzerland).

Inclusion criteria: Patients with AA determined by clinical findings (hair pull test, and dermoscopic findings). Patients with different degrees and types of AA, with ages above 18 years.

Exclusion criteria: Patients and controls having a personal or family history of infertility, diabetes mellitus, pregnancy, breastfeeding, congestive heart failure, endocrine dysfunction, malabsorption syndrome, chronic inflammatory disease, or connective tissue problem.

Ethical approval: The work was approved by the Medical Research Ethics Committee, NRC, Egypt. All participants provided written consents. Every phase of the study was conducted in accordance with the Helsinki Declaration.

Statistical analysis

The analysis was conducted using the SPSS statistical program version 28.0. The qualitative data was presented as numbers and percentages. The X^2-test was used to examine the relationship between the three groups' qualitative data. Quantitative variables were provided as median, mean ± SD and range. The independent t-test was used to compare two quantitative data sets. The ANOVA test was used to compare quantitative data across the three groups. Spearman correlation was used to determine the relationship between quantitative data. A P-value ≤ 0.05 was considered statistically significant.

RESULTS

The active AA group had 14 males (70%) and 6 females (30%). The ages varied from 21 to 46 years, with a mean of 30.75 ± 4.33 years. The non-active group consisted of 15 males (75%) and 5 females (25%). Their ages varied from 19 to 53 years, with an average of 31.55 ± 11.24 years. The control group consisted of 12 males (60%) and 8 females (40%), with ages ranging from 23 to 58 years and a mean of 34.40 ± 5.31 years. There was no statistically significant difference in sex (p=0.490) or age (p=0.288) among the three study groups (Table 1).

Table (1): Patient's characteristics of the studied groups

Variable			Active AA Group (n=20)	Non active AA Group (n=20)	Control group (n=20)	Tests	
						test	***P* value**
Age (years) Mean ± SD Range			30.75 ± 4.33 21-46	31.55 ± 11.24 19-53	34.40 ± 5.31 23-58	1.274 (f)	0.288
Sex	**Female**	**Number**	6	5	8	1.429 (x^2)	0.490
		Percent	30.0%	25.0%	40.0%		
	Male	**Number**	14	15	12		
		Percent	70.0%	75.0%	60.0%		

ANOVA test (f) Chi square test (X^2)

About 20% of active and 45 % of non-active cases had positive family history, and more than half of cases (70% in active and 55% in non-active group) showed patchy pattern of hair loss. Alopecia totalis (AT) affected 10% of either active or non-active cases, while alopecia universalis (AU) affected 20% of active and 35% of non-active cases. Eyebrow affection was found in 65% of active cases and in 40% of non-active cases. Nails were affected in 30% of active and 10% of non-active cases. Ophiasis was found in 20% of patients with active AA. Only one patient in the active group had atopic dermatitis. 45% of active cases and 85% of non-active cases had a history of steroids treatment either topical, intralesional or systemic, while 25% of active and 45% of non-active patients tried topical minoxidil. Diphenylcyclopropenon (DPCP) had been tried by 2 active AA patients and 5 non -active AA patients (Table 2).

Table (2): Clinical characteristics of the AA cases group (n=40)

Variables		Active AA		Non-active AA	
Duration(month)	Mean±SD	8.05±7.14			
	Median (IQR)	5 (2-13.75)			
Variable		**n=20**	**Percentage (%)**	**n=20**	**Percentage (%)**
Family history	Positive	4	20	9	45
	Negative	16	80	11	55
Pattern	Patchy	14	70	11	55
	Totalis	2	10	2	10
	Universalis	4	20	7	35
Associations	No	11	55	3	15
	Eyebrows	13	65	8	40
	Ophiasis	4	20	0	0
	Nail affection	6	30	2	10
	Atopic dermatitis	1	5	0	0
Previous treatment	No	6	30	0	0
	DPCP	2	10	5	25
	Steroids	9	45	17	85
	Minoxidil	5	25	9	45

When alopecia areata patients' serum calprotectin levels were evaluated, the mean level was 35.12 ± 4.87 ng/ml, and among control subjects (n=20) was 33.20 ± 2.28 ng/ml. Despite the alopecia areata patients had a higher mean level of serum calprotectin, there was no significant difference between both groups (Table 3).

Table (3): Serum calprotectin level of the studied groups

Serum calprotectin (ng/ml)	Cases Group (n=40)	Control group (n=20)	(t) test	P value
Mean ± SD	35.12 ± 4.87	33.20 ± 2.28	1.672	0.100

Independent t-Test (t)

On comparing serum calprotectin levels of active, non-active cases and the control group, the non-active AA group had higher mean levels than the normal subjects group, and the active AA group had higher mean levels than that of both groups. However, there was no statistically significant difference between the serum levels of the three groups (Table 4).

Table (4): Serum calprotectin levels among the studied groups

Serum calprotectin (ng/ml)	Active AA Group (n=20)	Non-active AA Group (n=20)	Control group (n=20)	tests	
				test	P value
Mean ± SD	35.27 ± 5.71	34.97 ± 3.99	33.20±2.28	1.402 (f)	0.254

ANOVA Test (f)

When serum calprotectin levels in the different patterns of alopecia areata were compared, we noticed that there was no statistically significant difference regarding the pattern of AA (Table 5).

Table (5): Comparing serum calprotectin levels in different patterns of the alopecia areata studied group

Pattern	Serum calprotectin	test	P value
Patchy	34.98 ± 5.61	-0.229 (t)	0.820
Totalis	35.84 ± 1.05		
Universalis	35.35 ± 3.46		

Independent t-Test (t)

There was no significant correlation between serum calprotectin level and either age of the patients or duration of the disease among alopecia areata cases (Table 6).

Table (6): Correlation between serum calprotectin level and both age and duration among alopecia areata cases

Variables		Calprotectin
Age	r	-0.066
	p	0.685
Duration	r	-0.310
	p	0.052

P=Sig. (2-tailed), r= Correlation Coefficient.

DISCUSSION

AA is an autoimmune, chronic, recurrent condition that causes patchy, non-cicatracial hair loss in hair-bearing regions [25]. It is an inflammatory condition that causes inflammation surrounding hair follicles in the anagen stage, resulting in the release of many bioactive chemicals such as inflammatory cytokines and ROS [26].

For a long time, fecal calprotectin levels have been utilized to assess disease activity in patients with gastrointestinal symptoms, especially IBD. Fecal calprotectin may act as a good indicator of gastrointestinal mucosal improvement [27]. Calprotectin can also be measured in serum in addition to feces [22]. Serum calprotectin is a promising inflammatory biomarker that may be helpful in monitoring the course of various autoimmune and inflammatory diseases as well as the effectiveness of treatment [28].

This study examined serum calprotectin value as an inflammatory biomarker in AA. Compared to volunteers, AA patients had mean serum calprotectin levels that were greater, but this difference was not statistically significant. Additionally, it was shown that there was no association between the disease activity and serum calprotectin. As far as we are aware, this is the first study to show a link between serum calprotectin and disease activity in alopecia areata.

Studies examining the relationship between calprotectin levels and disease activity in alopecia areata patients are scarce, although comparable investigations have been carried out in individuals with other inflammatory and autoimmune diseases. According to a research by **Duran *et al.* [29],** ankylosing spondylitis patients' blood calprotectin levels did not differ statistically significantly between patients and controls.

Many researches attempted to establish a relationship between the activity or severity of certain dermatological illnesses and the level of calprotectin. Serum calprotectin was tested in patients with psoriasis, atopic dermatitis, and healthy controls in a study conducted by **Aochi *et al.* [30]** when comparing patients with psoriatic arthritis and pustular psoriasis to healthy controls and those with atopic dermatitis, calprotectin levels were higher in these groups. However, calprotectin levels and the severity of atopic dermatitis were linked in a study by **Ali *et al.* [24]**.

In a study on acne vulgaris patients, serum calprotectin was correlated with acne severity and scar formation [31].

Some researchers studied the relation between markers of inflammation and AA. **Mustafa *et al.* [32],** reported increased CRP levels in AA patients, while **Gao *et al.* [33]** and **Yousefi *et al.* [34]** founded that there was no significant difference between CRP levels of AA patients and healthy controls.

Erythrocyte sedimentation rate (ESR) is another marker of inflammation that was analyzed by **İslamoğlu and Demirbaş [35]**, they unexpectedly founded that ESR was significantly lower in alopecia areata patients than controls.

In a study performed by **Gao *et al.* [33],** they founded that the levels of T3, T4, TPOAbs, ANA, CRP, and 25(OH) vitamin D were significantly correlated with AA. On the other hand they didn't find clear association between alopecia areata and serum TSH, TGAbs, C3, C4, IgA, IgM, and IgG levels.

CONCLUSION

Patients with AA had a higher mean level of serum calprotectin, but there was no significant difference between AA and healthy people. Although mean serum calprotectin level was higher in patients with active AA, it was not a predictor for activity in alopecia areata patients. Since our study had a limitation, which was the relatively small number of patients. Further prospective bigger investigations are needed to reveal the complete picture of serum calprotectin level changes during the different periods of AA disease and within its distinct patterns.

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