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Serum Interleukin 17 as a Marker for Alopecia Areata Severity: A Case-Control Study

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ABSTRACT

Background: Alopecia areata (AA) is a non-scarring skin disease affecting the scalp or other body parts. The etiology of AA is still controversial and various pathogenic causes are under investigations. It is characterized by infiltration of T helper 17 cells the hair follicles and chemokine secretion.

Objectives: The aim of the current work was to measure serum level of IL-17A in patients with AA and healthy controls to demonstrate if it has a role in the pathogenesis of A.A. and to correlate disease severity with its level.

Patients and methods: This case control study included a total of 26 A.A. patients, and 26 age and gender matched healthy controls, attending at Department of Dermatology, Venereology and Andrology, Faculty of Medicine, Ain-Shams University Hospitals, and Department of Dermatology, Venereology and Andrology, National Research Centre, Egypt. Serum level of interleukin-17 was measured. Clinical type, and the severity of the disease were determined for each A.A. patient.

Results: Serum level of interleukin-17 was significantly higher in A.A. cases compared to unaffected controls. The interleukin-17 level among A.A. patient was ranged from 133 to 326 (med. 195) pg /ml, while among controls, the level was ranged from 14 to 226 (med. 48) pg /ml.

Also, no statistically significant differences were found between interleukin-17 levels regarding the clinical types, age, gender, disease duration, and severity.

Conclusion: The significantly high concentration of serum interleukin 17 in A.A. patients supports the theory of its contribution in the process of the hair follicular destruction. It could also be concluded how beneficial its inhibitors can be especially in resistant cases of A.A.

Keywords: Alopecia areata, Hair follicle, Interleukin 17.

INTRODUCTION

Alopecia areata is the most prevalent autoimmune disorder and the second most frequent disease-causing hair loss after androgenetic alopecia (AGA) **(1).** Alopecia Areata (A.A.) is an auto-immune disease where T-cell infiltration and secretion of chemokines surrounding the hair follicles at their Anagen stage **(2)**. A.A. shows no gender or ethnic predilection **(3).**

Many theories for the pathogenesis developed, such as infection, a trophoneurotic hypothesis (based on emotional or physical stress and/or trauma), thyroid diseases and other hormonal imbalance (as in pregnant women or menopause) **(4).** AA is an autoimmune disease where the CD+8 cells attack the hair follicles as they already lost their immune privilege **(5).**

Exclamation point hair (A type of dystrophic hair), is a key characteristic of AA. Peri-bulbar inflammation is noticed surrounding the anagen follicles nearby the focal lesion **(6).** Alteration of one of the follicular growth phases could be the cause of A.A., a pre-mature transition of the anagen hair to the catagen one, or the anagen to telogen phase may happen, but it needs predisposing environmental, genetic, and immunologic factors to develop**(7).** There is increase in clonality in A.A. patients, which may suggest a common antigen epitope like trichohyalin peptides in cases of A.A**(8)**. Interleukin 17 (IL 17) is an inflammatory chemokine secreted mainly by Th17 cells and other cells like neutrophils, natural killer cells and C.D 8^+ T cells. Recently, the interest of finding the role of T cells in the development of autoimmune disorders increased and new targeted drugs on T cell show very promising efficacy in many auto-immune diseases like psoriatic patients**(9).** Various dermoscopic features detected in AA are black dots (cadaverous hairs), yellow dots, exclamation mark hairs and broken hairs**(10)**. Folliscope usage offers significant diagnostic tool in assessing grade of alopecia and grade of response to treatment. Folliscope allows accurate assessment of hair and skin changes in different types and phases in alopecia patients **(11).**

We measured interleukin-17 in serum of A.A. cases and healthy controls and compared these levels to demonstrate if it has a role in the pathogenesis of A.A. and also to correlate disease severity with its level.

PATIENTS AND METHODS

This case control study included a total of 26 A.A. patients, and 26 age and gender matched healthy controls, attending at Department of Dermatology, Venereology and Andrology, Faculty of Medicine, Ain-Shams University Hospitals, and Department of Dermatology, Venereology and Andrology, National Research Centre, Egypt.

Inclusion criteria: Patients with various degrees of alopecia areata who had not received systemic or topical treatment in at least 1 month. Both sexes were included.

Exclusion criteria: Patients with other dermatological, autoimmune, or systemic diseases like rheumatoid

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arthritis, psoriasis, inflammatory bowel disease, pregnant or lactating patients, and patients receiving immunosuppressive drugs were excluded.
The control individuals were selected from the healthy individuals attending the clinic such as patients' relatives.

All patients were subjected to the following: Personal history taking including name, age. Past history of similar lesions (number, site, duration, previous treatment and any side effects). Medical history of systemic diseases, dermatological diseases, and any autoimmune diseases e.g., diabetes mellitus, vitiligo, atopic dermatitis, or thyroiditis. Family history of similar condition. Complete general and dermatological examination including nails for any changes and scalp for evidence of inflammation, scales, erythema and scarring. Local scalp examination was done to confirm diagnosis and for assessment of disease severity.
Grading of scalp affection was done according to the SALT score: (S.0 = no scalp hair loss, S.1 = <25%, S2 = 25-49%, S3 = 50-74%, S4 = 75-99%, a = 75-95%, b = 96-99%, S5 = 100% hair loss) [7]**.** The severity of scalp disease was classified as: (Mild (S1, S2), Severe (S3, S4, S5)[12]**.**

Specimen collection:

Blood samples were taken from patients, sera were separated and stored at –80° C until measuring the IL 17 level by enzyme-linked immunosorbent assay (ELISA)

Ethical consent

The study was authorized by Ain Shams University's Ethical Institutional Review Board. All study participants provided written informed consent after being aware of the research's goals. The study protocol conformed to the Helsinki Declaration, the ethical norm of the World Medical Association for human testing.

Statistical analysis

Data were collected, processed, coded, and entered into IBM SPSS version 23's Statistical Package for Social Science. When the quantitative data was parametric, it was presented as means, standard deviations, and ranges; otherwise, it was presented as medians and interquartile ranges (IQR). Qualitative aspects were also statistically and as percentages expressed. To compare the quantitative data of the two groups with parametric and non-parametric distributions, the Independent t-test and Mann-Whitney test were used.
The Kruskall-Wallis test was used to compare non-parametric quantitative data across multiple groups. To test the link between two numerical parameters within the same group, Spearman correlation coefficients were used. A P value of 0.05 or less was considered significant.

RESULTS

This study included 26 patients and 26 controls. Among the 26 patients, 17 were males (65.4%) and 9 were females (34.6%) with ages ranging from 9.5-61 years (mean 32.10 ± 13.13 years). The control group was 26 healthy age and sex-matched subjects, 16 were males (61.5%) and 10 were females (38.5%) with ages ranging from 12-65 years (mean 38.19 ± 14.88 years).

The duration of the disease among cases ranged from 0.1 months (3 days) to 204 months (17 years) with a median of 6.5. Regarding the clinical type, 21 cases (80.8%) were patchy, 4 cases (15.4%) ophiasis and 1 case (3.8%) was alopecia totalis. Regarding the sites affected (figure 1), 18 cases (69.2%) had only scalp affection, 3 cases (11.5%) had beard affection, 3 cases (11.5%) had both scalp and beard affection and 2 cases (7.7%) had both scalp and eyebrows affection. The number of patches ranged from 1-30 with a median of 3 (Figure 1).

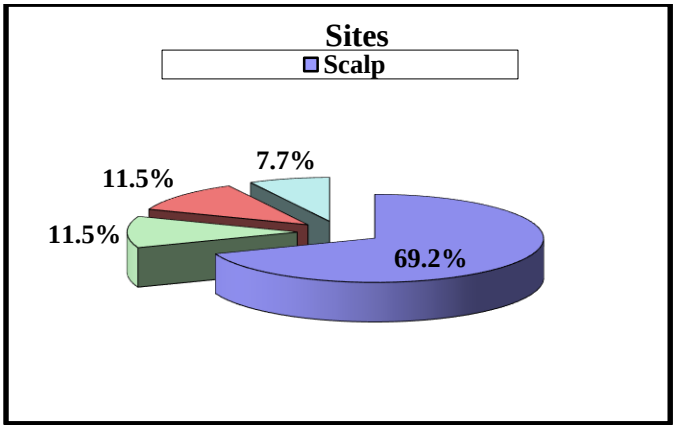

Figure (1): Sites affected.

The SALT score ranged from 0-96 with a median of 4. As regards SALT subclasses, 3 cases (11.5%) were S0, 19 cases (73.1%) were S1, 1 case (3.8%) was S2, 2 cases (7.7%) were S3 and 1 case (3.8%) was S4 (table 1) As regards the severity of the disease according to the SALT subclass, 20 cases (76.9%) were mild and 3 (11.5%) cases were severe (Table 1).

Table (1): Description of disease characteristics among cases

		Cases group
		No. = 26
SALT score (%)	Median (IQR)	4 (2 - 14)
	Range	0 – 96
SALT subclass	S0	3 (11.5%)
	S1	19 (73.1%)
	S2	1 (3.8%)
	S3	2 (7.7%)
	S4	1 (3.8%)
Severity	No	3 (11.5%)
	Mild	20 (76.9%)
	Severe	3 (11.5%)

As regards treatment status, 18 cases (69.2%) had stopped treatment with a range of 1- 84 months prior to our study and 8 cases (30.8%) hadn't received treatment at all.

As regards the type of treatment received, 13 cases (50%) received ILCS only, 1 case (3.8%) received minoxidil only, 1 case (3.8%) received both ILCS and minoxidil and 3 cases (11.5%) received unknown treatment. The IL 17 level among A.A. patients ranged (from 133.38 to 325.99) pg/ ml (med.195.38), while among controls, levels ranged (from 13.59 to 225.73) pg/ ml (med. of 48.42). There was a high statistically significant differences between cases and controls regarding IL 17 levels with higher IL 17 levels among cases as compared to controls (Table 2 and Figure 2).

Table (2): Levels of IL 17 in cases and controls

IL 17 Concentration pg/ml	Control group No. = 26	Cases group No. = 26	Test value‡	P value	Sig.
Median Range	48.42 (23.09 – 99.60) 13.59 – 225.73	195.38 (172.96 – 241.56) 133.38 – 325.99	-5.885	0.000	HS

Median, Range: Non-parametric test. ‡: Mann Whitney test

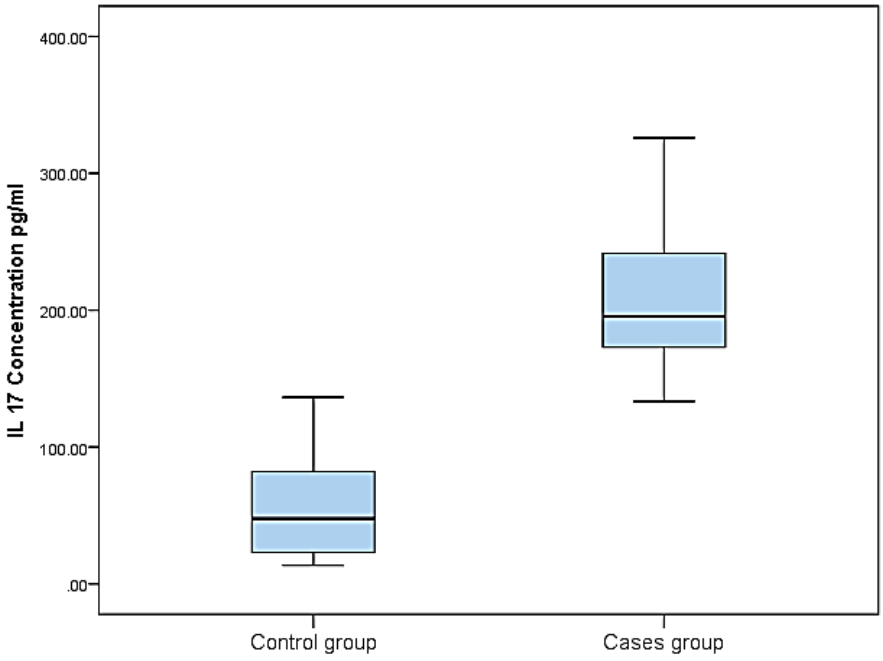

Figure (2): Cases and control group regarding IL-17.

No statistically significant differences were found in IL 17 concentrations regarding number of patches, duration of stoppage of treatment, disease duration, or S.A.L.T. score (Table 3).

Table (3): Correlation between age, duration of disease, number of patches, SALT score and duration of stoppage of treatment regarding IL-17

	IL 17 Concentration pg/ml	
	r	P-value
Age (Year)	-0.084	0.682
Duration	0.107	0.603
Number of patches	-0.010	0.961
SALT score (%)	0.187	0.360
Duration of stop (months)	0.321	0.194

Also, no statistically significant differences were found in IL 17 concentrations regarding clinical type, the affected sites, age, sex, SALT sub-classes, severity, treatment status, or the type of treatment used (Table 4).

Table (4): Correlation between sex, clinical type, sites of affection, SALT subclasses, severity, treatment status and treatment taken regarding IL-17 concentration among cases

		IL 17 Concentration pg/ml		Test value	P-value	Sig.
		Median(IQR)	Range			
Sex	Female	238.92 (186.15 – 244.20)	143.93 – 304.88	-1.186ǂ	0.235	NS
	Male	186.15 (172.96 – 233.64)	133.38 – 325.99			
Clinical type	Patchy	191.42 (175.59 – 241.56)	133.38 – 304.88	1.651ǂǂ	0.438	NS
	Ophiasis	237.60 (196.70 – 282.46)	157.12 – 325.99			
	Totalis	159.76 (159.76 – 159.76)	159.76 – 159.76			
Sites	Scalp	231.01 (184.83 – 244.20)	136.02 – 325.99	6.166ǂǂ	0.104	NS
	Beard	172.96 (133.38 – 186.15)	133.38 – 186.15			
	Scalp- Beard	233.64 (183.51 – 241.56)	183.51 – 241.56			
	Scalp- Eye Brows	156.60 (153.43 – 159.76)	153.43 – 159.76			
SALT grade	S0	172.96 (133.38 – 186.15)	133.38 – 186.15	5.257ǂǂ	0.262	NS
	S1	199.34 (175.59 – 244.20)	136.02 – 325.99			
	S2	238.92 (238.92 – 238.92)	238.92 – 238.92			
	S3	240.24 (238.92 – 241.56)	238.92 – 241.56			
	S4	159.76 (159.76 – 159.76)	159.76 – 159.76			
Severity	No	172.96 (133.38 – 186.15)	133.38 – 186.15	2.864ǂǂ	0.239	NS
	Mild	212.54 (179.55 – 242.88)	136.02 – 325.99			
	Severe	238.92 (159.76 – 241.56)	159.76 – 241.56			
Treatment Status	Stopped	208.58 (159.76– 238.92)	133.38 – 325.99	-0.417ǂ	0.677	NS
	Not taken	192.75 (179.56 – 242.88)	157.12 – 291.69			
Treatment Taken	None	192.75 (179.56 – 242.88)	157.12 – 291.69	3.569ǂǂ	0.467	NS
	Unknown	183.51 (153.43 – 238.92)	153.43 – 238.92			
	ILCS	191.42 (159.76 – 236.28)	133.38 – 325.99			
	Minoxidil	304.88 (304.88 – 304.88)	304.88 – 304.88			
	ILCS & Minoxidil	238.92 (238.92 – 238.92)	238.92 – 238.92			

Median, Range: Non-parametric test. *:Chi-square test; •: Independent t-test; ‡: Mann Whitney test.

DISCUSSION

Alopecia areata is non-scarring patches of complete hair loss affecting the scalp or other body parts. It is a common disease that affects 1 %- 2 % of population. Its etiology is unknown several treatment options for AA have been proposed including corticosteroids, topical minoxidil, anthralin and immunotherapy and biologics [13]. The pathogenesis is not well understood, but autoimmune theory of Th 17 lymphocytes is one of the important pathogeneses, but not fully established [6, 14]. Immune privilege loss in the same time infiltration of the T cells the hair follicles auto-antigens was proved in many studies [13-15]. The role of CD4 and CD8; Th cells which infiltrate the hair follicle in A.A cases; both have been shown to secrete interleukin-17[16]. We tried to evaluate the role of IL 17 in the pathogenesis of A.A. to be reflected in the treatment and follow-up of AA patients.

Our work represented a case control study where 52 subjects were recruited; 26 alopecia areata cases and 26 age and sex matched healthy controls. Serum interleukin-17 levels were measured by ELISA technique. Regarding the level of IL-17, all alopecia areata patients had significantly high IL-17 levels as compared to healthy controls. Our findings were consistent with **El-morsy *et al.*** [16], **A. Elela *et al.*** [17], **Atwa *et al.*** [18], and **Loh *et al.*** [19] who found that the mean serum levels of IL-17 were significantly higher in alopecia areata patients in comparison to controls. However, our study found no significant correlations between interleukin-17 levels and other factors like age, sex, disease duration, SALT score, or severity of the disease.

In contrast to our study, **Atwa *et al.*** [18] found a significant relation between blood concentration of interleukin-17, clinical type, and severity of A.A. This may be explained by the higher number of subjects in their studies or the variety in the clinical types.

Moreover in contrast to our study which found no correlation between age and IL-17 levels, **El-morsy *et al.*** [16] found that serum interleukin-17 A levels were significantly higher in younger patients than in old patients. This elevation in blood interleukin-17 -A level was even more significant in patients aged <15 years compared with patients aged >30 years. However, no correlation was found between IL 17 concentrations with sex, duration of A.A., SALT scoring, and severity of the disease which is consistent with our study. Psychological stresses may be the cause of higher levels of Interleukin-17 in young patients as this age group is more vulnerable to stress. There are many theories that prolonged stress can affect activated T cells and shift their pathway towards Th-17 response. Therefore, juvenile-onset A.A. has less favorable prognosis

compared to those whom developed A.A. at an older age**.** Also, **Loh *et al.* [19]** found a positive correlation between serum IL 17 and severity of the disease, his study was done on high number of cases with severe forms of AA; 34 had a patchy type, 12 had alopecia totalis/alopecia universalis and 8 had alopecia totalis.

A previous study assessed Th-17 cells in serum of more than one hundred patent and compared them to healthy controls. It was noticed that Th-17 cells were higher in patients compared to controls. Also, patients with long duration period showed lower number of Th-17 cells. The patients with active disease showed higher number of cells compared to those with stable A.A. However, there was no correlation between the number of Th 17 cells in serum and the severity of A.A [9, 20].

Moreover, **Tojo *et al.* [12],** stated that the cells which secrete IL 17 were found around the hair follicles in A.A. whereas, in psoriatic patients, they are mainly distributed in dermal papillae. He did immune-histochemical staining for Interleukin-17 and Interferon-γ from 17 cases of A.A. and found that Interleukin-17-secreating cells are present around the hair follicles, whereas Interferon-γ-secreting cells were mainly infiltrating the dermis. From this, we can conclude that the role of Interleukin-17 is stronger than interferon γ-secreting cells in the pathogenesis of the alopecia areata.

CONCLUSION

The significantly high concentration of serum interleukin 17 in A.A. patients supports the theory of its contribution in the process of the hair follicular destruction. It could also be concluded how beneficial its inhibitors can be especially in resistant cases of A.A. IL 17 has a major role in the pathogenesis of A.A., and this can be explained by the significant higher concentrations in serum of patients with A.A. Serum IL 17 level isn't influenced by the disease severity. Furthermore, age, sex, disease duration, and clinical type do not affect the serum level of IL-17.

RECOMMENDATIONS

Biological therapies including Interleukin-17 A inhibitors, can be recommended as a possible therapeutic modality in treatment of resistant cases of Alopeci areata.

Conflict of interest: None
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