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Assessment of Interleukin-17A and Interleukin-2 Serum Levels in Patients with Alopecia Areata

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ABSTRACT

Background: *Alopecia areata (AA) is a condition that leads to the loss of hair in the part of the body due to the destruction of hair follicles, and causing patchy, non-scarring hair loss. The source of AA is unclear; however, there is much evidence assuming that alopecia areata is a disease mediated by the immune system.* ***Aim:*** *To measure the levels of serum interleukin (IL-17A and IL-2) in alopecia areata patients and their associations with the severity and period of illness, along with other prognostic disease variables.* ***Materials and Methods:*** *To perform the study, 40 patients with alopecia areata and 40 healthy controls were registered. The serum levels of IL-2 and IL-17A in alopecia areata patients and healthy controls were evaluated by applying ELISA techniques (Sandwich ELISA).* ***Results:*** *Compared to the healthy group, patients with alopecia areata revealed much higher levels of IL-17A and IL-2 ($p > 0.05$). Baseline interleukin levels did not associate statistically significantly with the severity or period of the disease.* ***Conclusions:*** *The current study suggests that IL-17A and IL-2 were strongly linked with alopecia areata and may have a crucial role in the condition's progress.*

Keywords: Alopecia areata, IL-17A, IL-2, ELISA, Autoimmune.

Article Information

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INTRODUCTION

Alopecia areata (AA) is an immune-mediated disease that leads to non-scarring patchy hair loss (without inflammation) by damaging hair follicles [(1)]. When alopecia areata manifests clinically, it might exhibit one or more scalp patches of hair loss, whole baldness in the scalp (alopecia totalis), or full-body hair loss (alopecia universals) [(2)]. Around 2% of general people worldwide may have alopecia areata, regardless of age, sex, or ethnicity [(3)]. This disease is considered one of the most public causes of hair loss in people. The condition has significant psychological effects for both men and women, specifically about community acceptance and psychological well-being [(4)]. The precise etiopathogenesis of alopecia areata is still unidentified, but there are many proofs that assume that alopecia areata is a condition activated due to the destruction of the immune privilege of hair follicles by the immune system [(3)]. Alopecia areata are characterized by infiltrates of many of the cytokines and T cells in the hair follicles [(5)]. Many of the cytokines result in cause of hair loss due to interfering with the growth cycle of hair [(6)]. Some studies displayed the inhibition of follicular tolerance to the immune system in alopecia areata and are linked to irregularity and dysfunction of the

numerous subsets of T-lymphocytes involving Th1, Th2, Th17, and Regulatory cells (Treg) [7]. These cells are supposed to have produced cytokines that are potentially important in the pathophysiology of alopecia areata[8].

Activated T cells produce the interleukin known as IL-2, which was first discovered in 1976 [9]. It is a crucial mediator in the maturation, expansion, and function of natural killer cells, lymphokine-activated killer cells, and B and T lymphocytes [10]. Major Histocompatibility Antigen (MHC) and Interferon-γ expression are controlled by IL-2, which also motivates T-cell production, particularly to the antigens [10]. IL-2 may have different critical roles in immunity, tolerance, and other important immune system functions due to its direct effects on T cells. Cytokine IL-17, sometimes identified as IL-17A, is created by the CD4 Th17, NK cells, and CD8 cytotoxic cells[11].IL-17R is expressed in a numerous tissue, but on the hair follicle is not known yet [12]. The pathogenesis of many inflammatory and autoimmune diseases has been associated with IL-17A [13]. Increased production of IL-17A has been related to several chronic diseases like vitiligo, psoriasis, dermatitis, arthritic joints, lupus erythematosus, and bowel inflammation [12].

This study aims to evaluate the levels of serum IL-2 and IL-17A in alopecia areata patients and know how these levels are correlated with the severity, duration of disease, and other prognostic variables.

MATERIAL AND METHODS

Study Subject

In this study, a case-control analysis was done from the first of December 2022 to the first of April 2023. In the Al-Najaf province, the patients who are suspected of alopecia areata were identified clinically by physicians in dermatological specialists at Al-Sador Hospital and Al-Najaf Teaching Hospital. Patients with alopecia areata were personally interviewed by using an anonymous questionnaire form that addressed age, gender, drug history, family history, severity, duration of diseases, and recurrence of diseases.

The current study included 40 patients with AA (16 females, 24 males) and 40 healthy persons (14 females and 26 males) who had no history of systemic disease and were clinically considered healthy as a control group. Our inclusion criteria were patients with patchy AA of various degrees of severity.

Evaluation of IL-17A and IL-2

This study was conducted to estimate the levels of IL-17A and IL2 in the serum blood of patients with AA. About three milliliters of blood were transferred in a sterile gel tube and left to clot at room temperature for a few minutes to an hour. The serum was then centrifuged at 2500 rpm for 10 minutes before being reserved in Eppendorf tubes and freezing at -20 C until later used for immunological examinations by the ELISA test (Sandwich method). The kits used for IL-2 were (Solarbio) and for IL-17A is (Sunlong) and the tests were performed according to the instruction manufacturer. At 450 nm the absorbance was assessed.

Statistical analysis

The data were shown as means ± standard deviation. This study involved the P-value, T-test, ROC curve, Chi-square, and correlation for the statistical analysis.

Ethical approval

The local ethics committee assessed the study protocol, subject details of the study, and permission form; based on text number 6210, dated 2023/2/9, the committee accepted the study.

RESULTS

The present study involved 40 patients with alopecia areata and 40 healthy controls. The median age of patients with AA was 27.80 ± 12.64 years, whereas that of control participants was 25.80 ± 5.69 years. Between patients and control groups, there was no statistically significant difference in mean age (P= 0.364). The patients group included 16 (40.0%) females and 24 (60.0%) males, whereas the control group included 14 (35.0%) females and 26 (65.0 %) males. The frequency distribution between the patients with AA and control groups did not differ statistically significantly based on gender (P=0.644). The frequency distribution of patients with AA and control participants did not change significantly based on age (P = 0.118). The demographic features of both healthy control and alopecia areata patients are displayed in Table 1. The frequency distribution of patients with alopecia areata, based on family history, was as follows: of the patients with alopecia areata, 32 (80.0%) have a negative family history, and 8 (20.0%) have a positive family history, Figure (1). Compared to healthy controls, alopecia areata patients had significantly greater levels of IL-17A and IL2 in serum (P < 0.001), Table (2). This study used Receiver Operator Characteristic (ROC) curve analysis to estimate the cut-off of IL-17 and IL-2 in patients with AA and the results revealed the best cut-off for IL-17A was more than 10.74 with the area under the curve was 0.991 (0.978-1.000). Also, the best cut-off for IL-2 was greater than 486.04 and the area under the curve was 0.647 (0.522- 0.773). Depending on the available data IL-17A considered was a good diagnostic marker and IL-2 a week diagnostic marker, more details of results are displayed in Table (3). The present results showed that IL-17A and IL-2 levels were significantly higher in patients with more than 4 years of duration in comparison to other groups. Also, the present results show a non-significant difference in IL-2 and IL17-A levels according to recurrence of disease and severity (P< 0.05). Additionally, a non-significant difference in IL-2 and IL-17A levels was seen between patients with a positive history of drug use and those with a negative history, and patients with a positive family history had non-significantly greater mean levels of IL-17A and IL-2 than patients with a negative family history, Table (4).

Table (1): The demographic characteristics of individuals with alopecia areata and healthy controls.

Characteristics	Patients *n* = 40	Healthy control *n* = 40	*P*
Age (years)			
Mean ±SD	**27.80 ± 12.64**	**25.80 ± 5.69**	**0.364 NS**
Range	**5 –65 years**	**18– 40 years**	
< 20, n (%)	**10 (25.0%)**	**7 (17.5%)**	**0.118**

Characteristics	Patients $n = 40$	Healthy control $n = 40$	*P*
20-29, *n* (%)	11 (27.5%)	20 (50.0%)	NS
≥ 30, *n* (%)	19 (47.5%)	13 (32.5%)	
Gender			
Male, *n* (%)	24 (60.0 %)	26 (65.0 %)	0.644 NS
Female, *n* (%)	16 (40.0%)	14 (35.0%)	

n: Number of partecipents; SD: Standard deviation; †: Independent cases t-test; ¥: Chi-square test; NS: not significant at P > 0.05.

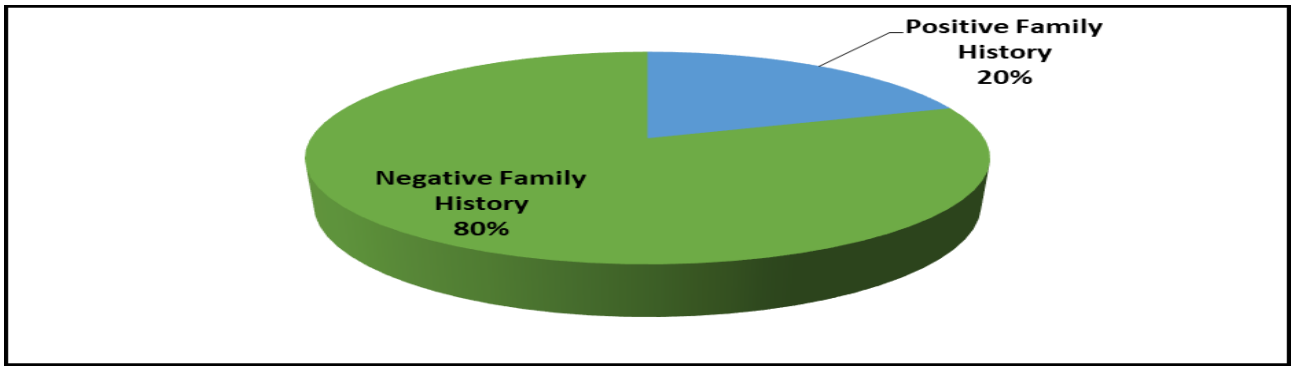

Figure (1): Alopecia areata patients' distribution based on family history.

Table (2): IL-17A and IL2 levels in patients with Alopecia areata and healthy control.

	Cases –control comparison		
	Patients $n = 40$	Healthy control $n = 40$	*P*
IL-17A levels			
Mean± SD	15.84 ± 3.22	4.95 ± 2.51	< 0.001 † HS
Range	10.49 – 22.38	0.21-12.29	
IL2 levels			
Mean± SD	559.19 ± 195.71	401.11 ±69.07	0.004 † S
Range	214.74 – 1184.78	97.36-600.23	

SD: standard deviation; n: number of cases; ¥: Chi-square test; †: independent samples t-test; NS: not significant at P > 0.05.

Table (3): Receiver Operator Characteristic (ROC) curve analysis of IL17A and IL-2.

Variables	Cut-off	Sensitivity	Specificity	PPV	NPV	AUC (95% CI)
IL17-A	≥ 10.74	95%	95%	95%	95%	0.991 (0.978-1.000)
IL-2	≥ 486.04	65%	67.55	65.9%	66.7%	0.647 (0.522- 0.773)

CI: Confidence interval, AUC: Area under curve.

Table (4): Frequency distribution of IL-2 and IL-17A levels according to some characteristics.

Characteristic	IL-17A	IL2
Duration of disease		
< 1 years n=19	17.41 ± 4.98	543.78 ± 211.97
1-3 years n=17	14.37 ± 3.31	513.74 ± 183.72
≥ 4 years n=4	15.32 ± 3.09	832.65 ± 37.05
P-Value	0.101 A NS	0.142 A NS
Severity of Disease		
Mild n = 16	14.86 ± 3.34	461.24 ± 163.54
Low n = 15	17.49 ± 5.40	677.92 ± 129.64
High n = 9	18.82 ± 3.16	697.82 ± 160.31
P-Value	0.172 A NS	0.243 A NS
Family history		
Positive n = 8	15.92 ± 4.16	576.56 ± 219.03
Negative n = 32	15.61 ± 4.31	554.15 ± 193.98
P-Value	0.938 † NS	0.844 † NS
Drug history		
Positive n = 20	16.11 ± 4.01	547.23 ± 189.39
Negative n = 20	15.56 ± 4.29	571.15 ± 208.94
P-Value	0.692 † NS	0.802 † NS
Recurrence of disease		
Positive n =11	15.99 ± 3.06	574.06 ± 197.37
Negative n = 29	15.78 ± 4.73	519.99 ± 201.95
P-Value	0.889 † NS	0.612 † NS

n: Number of particepents; SD: standard deviation; †: Independent cases t-test; A: one way anova test;; NS: not significant at P > 0.05

DISCUSSION

Few studies investigate the correlation between the cytokine in progress of alopecia areata. In our study, selected IL2 and IL-17A to evaluate this interleukine in the progress of alopecia areata. The hematological parameter has been regarded as a diagnostic biomarker for many skin diseases like psoriasis and vitiligo [14].

Alopecia areata (AA) is a disease that leads to loss of hair in the part of the body and mostly occurs in the scalp of the head. Other, regions of the body that are affected include eyebrows, eyelashes, moustache, and beards [15]. Alopecia areata is a disease that occurs due to the immune response against auto-antigen in the hair follicle and leads to form oval patches without scarring [16]. Sometimes, the definitive diagnosis of alopecia areata is difficult and treatments are limited in progress diseases. The etiopathogenesis of alopecia areata is not yet fully known, but much of the evidence suggests that immunological responses may break down the immune privilege of hair follicles [3].

IL-2 is an interleukin that is created by activated T cells, which was first identified in 1976 [9]. IL-2 is crucial for T and B lymphocytes and NK cells to develop and activate [10]. The serum of IL-2 levels has been changed in various conditions, like vitiligo, psoriasis, and lupus erythematosus [17]. The serum levels of IL-2 concentrations for some of these disorders were found to be linked to disease activity and intensity and may be used as a prognostic indicator. Because it has a crucial role in the immune response, IL-2 is considered an important molecule for therapeutic and diagnostic applications [10].

In the current research, IL-2 was selected as the cytokine to assess its influence on the progress of alopecia areata. Patients with alopecia areata exhibited significantly higher serum levels of IL-2 than healthy groups ($P = 0.004$). Little studies have been done on the serum levels of this cytokine in patients with alopecia areata, so the present investigation focused on IL-2.

Studies conducted by Kasumagi´c-Halilovic et al., (2018); Sharma and Tembhre (2013); Askın et al., (2021) demonstrated alopecia areata patients had a higher serum level of IL-2 than healthy controls, and these results were consistent with the study results. Also, a study conducted by Aljabali and Kuts., (2022) showed an increase in IL2 as compared to healthy control, and these results agreed with the study results. Conversely, Loh et al., (2018) showed that blood IL-2 levels in alopecia areata patients were lower than those in healthy controls.

The present results found there was a non-significant link between IL-2 level and length of disease ($p = 0.142$), family history ($p = 0.844$), drug history ($P= 0.802$), and Recurrence of disease ($p = 0.612$). However, the study by Tembhre et al., (2013) indicated a positive relationship between the serum IL-2 level and the total duration of the disease, and these results disagree with the results of the study. Furthermore, a survey conducted by Kasumagić-Halilovic et al., (2018) and Askın, et al.,(2021) found there was no significant link between the concentration level of IL2 and the length of the disease, and these results agree with the results of the study.

IL-17A is a cytokine generated by cytotoxic and Th17 cells [20]. Some of the pro-inflammatory cytokines, like IL-1, IL-6, and TNF-alfa, are created by the monocyte cells due to induced by IL-17A [21]. IL-17A can be increased or produced in many autoimmune diseases, including skin diseases like psoriasis and vitiligo.

The present study focused on IL-17A to assess its impact on the progress of the alopecia areata. The outcomes of the results revealed there was a significant change ($P < 0.001$) between alopecia areata patients and healthy

controls. Many studies revealed that patients with alopecia areata had higher levels of IL-17A in comparison to healthy controls [17]. Furthermore, the studies that are conducted by Atwa et al., (2016) Wojciechowska-Zdrojowy et al .,2021 and El-Morsy et al ., 2016 show there were significantly elevated serum levels of IL-17A in patients with alopecia areata than the healthy groups. These results are comparable with the results of the study.

The current results displayed a non-significant relationship between the period of the disease and the IL17A level (p= 0.101), drug history (0.692), family history (p= 0.938), and the recurrence of disease (p= 0.889). The Study conducted by El-Morsy et al., 2016 found the correlation between the severity and duration of disease with serum IL-17A levels was non-significant, and these results agree with the results of the study. Also, the studies done by Askın et al., (2021) and Ramot et al ., 2018 found the length of the disease or its severity did not significantly correlate with IL-17A levels, and these results agree with the results of the study. However, a study conducted by Atwa et al., (2016) found significant positive correlations between serum IL-17 and disease severity and these results disagree with the results of the study.

CONCLUSIONS

The present study's conclusions that IL-2 and IL-17A levels were remarkably higher in patients with alopecia areata than in healthy groups, indicating that these cytokines may have a crucial role in the pathophysiology of AA.

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