The Egyptian Journal of Hospital Medicine (January 2022) Vol. 86, Page 831-836

Serum IL-21 Level and Its Relation to Activity and Severity of Alopecia Areata

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ABSTRACT

Background: Alopecia areata (AA) is a non-cicatricial alopecia that is postulated to be a hair-specific autoimmune disease, with genetic factors playing a role in disease susceptibility and severity. The disease presentation ranges from circular patches on the scalp to complete hair loss with devastating psychosocial consequences.

Patients and methods: This was a case control study carried out on 40 patients diagnosed as alopecia areata. They were recruited from the outpatient clinic of Dermatology, Andrology and STDs Department, Mansoura University Hospitals. In addition 40 normal healthy subjects with matched age and sex were selected to act as a control group.

Results: Serum levels of IL-21 were significantly increased in AA patients, and showed significant positive correlation with activity of the disease. Higher serum levels of IL-21 in active cases support its role as predictor of disease activity. There were no significant differences in IL-21 level with different SALT scores. Receiver Operating Characteristic (ROC) curve analysis of IL-21 was conducted to evaluate the sensitivity and specificity of serum IL-21 as a diagnostic index for AA. The AUC-ROC of IL-21 was excellent (0.962); and the best cut off point for IL-21 was determined to be 22.22 pg/ml. It was good predictive value. Its accuracy was 86.3%.

Conclusions: The results of this study indicate that the serum IL-21 could be promising marker in the diagnosis of alopecia areata, and also can be used as prognostic marker of its activity.

Keywords: Alopecia areata, Serum IL-21 level.

INTRODUCTION

Alopecia areata (AA) is a non-cicatricial alopecia that is postulated to be a hair-specific autoimmune disease. Several genetic factors play role in disease susceptibility and severity. This condition is a relatively common cause of hair loss, without demonstrated predilection for gender or age [(1)].

Alopecia areata presentation includes round or oval patches of non-scarring hair loss. At the margins of areas of alopecia, short "exclamation point" hairs (i.e. distal end broader than the proximal end) can often be seen. Other presentations include alopecia totalis (loss of all scalp hair), alopecia universalis (loss of all scalp and body hair), an ophiasis pattern (band-like pattern of hair loss along the periphery of the temporal and occipital scalp), and alopecia involving the beard area. In addition to the hair follicle affection, nails may be involved [(2)].Trichoscopy should be done during evaluation of the patient to allow for evaluation of the follicle, hair shaft, and surrounding skin. The clinician should examine for exclamation point hairs, which are pathognomonic indicator of AA [(3)]. The bad prognostic signs include association with atopy, ophiasis, affection of eye brow, nail changes, and presence of multiple exclamation marks [(4)].

There is an increased frequency of other autoimmune disorders in patients with alopecia areata compared to the general population [(4)]. The proximal portion of the anagen hair follicle (HF) is an immune privileged site [(5)]. The hair follicle immune privilege is disrupted in AA, with increase in major histocompatibility complex (MHC) I and II molecules. These changes increase the antigens presentation by HF cells and enhance T cells migration to close proximity of HF in AA lesions [(6)].

Histological features of the disease include perifollicular and intrafollicular cellular infiltrates consisting predominately of CD4+ and CD8+ T cells. This infiltrate surround anagen follicles, with extension into the hair matrix keratinocytes. The lymphocytes accumulation results in disorganization and apoptosis of hair matrix cells. In chronic alopecia areata, the majority of follicles are in telogen phase [(7)].

The immune attack in AA spares the stem cell compartment, preventing permanent organ destruction. The future regrowth remain possible in most cases [(8)]. The involvement of autoreactive T cells rises the hypothesis of HF-derived autoantigens [(9)].

Cytokines are immunomodulators, which mediate inflammation and regulate cell proliferation. Cytokines have a significant pathogenic role in AA [(10)]. T helper 17 (TH17) has become a focus of current immunology in association with autoimmune disease .The maturation of it needs the stimulation of naïve T cell by both TGF and IL-21 [(10)]. Interleukin-21 is a cytokine that is produced mostly by activated CD4+ T cells. It regulates the function of immune and non-immune cells. It controls the differentiation and activity of T cells, B cells and NK cells. It limits the differentiation of regulatory T cells (Tregs), and makes

Received: 16/09/2021
Accepted: 13/11/2021

T cells resistant to the Treg-mediated immunosuppression [(11)].

The aim of this work was to evaluate of IL-21 serum level in alopecia areata patients in comparison to healthy control group, and studying the relation between it and the disease activity and severity.

PATIENTS AND METHODS

This was a case control study carried out on 40 patients diagnosed as alopecia areata. They were recruited from the outpatient clinic of Dermatology, Andrology and STDs Department, Mansoura University Hospitals. In addition, 40 normal healthy subjects with matched age and sex were selected to act as a control group.

Ethical considerations:

This study was approved by the Institutional Research Board (IRB) of Mansoura University Faculty of Medicine (IRB code MS.19.03.529). All patients signed written informed consent before participation in the study. This work has been carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Inclusion criteria: Patients of both sexes with active and stable alopecia areata diagnosed by clinical features and confirmed by trichoscopy, age of patients was 18 – 50 years, and control group of healthy people without any previous history of autoimmune disease with matched age and sex.

Exclusion criteria: Other differential diagnoses of alopecia areata (Trichotillomania, temporal triangular alopecia, telogen effluvium, and tinea capitis), patients on corticosteroids or other immunosuppressive therapy, patients with immunodeficiency, patients who were recently experiencing significant spontaneous regrowth of terminal hair, and patient refusing participation in the study.

All patients were subjected to the following:

I) History:

A) Personal history: Name, age, sex, occupation, medical problems and special habits of medical importance.

B) Present history: A detailed history taking were obtained from each patient concerning the following: Current episode of hair loss, site, onset, course and duration, Pain, burning sensation or paresthesia in the scalp, recent associated autoimmune disease, Atopy, drugs (dose, duration), exposure to radiation, and changes in the nails.

C) Past history: Prior history of alopecia areata (number of prior episodes), history of alopecia totalis or universalis, any disease of medical importance, and drug (dose, duration).

D) Family history: Similar conditions in the family.

II. Physical examination:

All patients were subjected to full clinical examination that included:

1. General Examination: Full general examination.

2. Dermatologic Examination:

A) Scalp examination: Hair Examination: Hair shaft examination for any abnormalities, scalp examination for any signs of inflammation or infection, determination of hair loss pattern (patchy, ophiasis or alopecia totalis), and assessment of disease severity.

B) Body examination: The whole body hair was examined.

C) Nail involvement: Nails were examined for any changes.

D) Dermoscopic examination: The alopecia areata was examined by dermoscopy for signs of alopecia areata.

E) Assessment of activity:

1. Hair pull test: (a) Fifty to sixty hairs were selected and held between thumb, index finger and middle finger. Then gently pull using slow traction. It was considered positive if more than 10% of pulled bundle were removed. (b) It was done at the periphery of alopecia areata and in other non-affected areas.

2. Dermoscopic signs of activity: Exclamation mark hair, and black dots.

3. Laboratory investigation:

Sampling:

Three ml of venous blood collected from all patient and control groups by clean venipuncture using plastic disposable syringes. The blood was delivered into plain collecting tube. Blood were allowed to clot for 10 to 20 minutes and then centrifugation for 10 minutes at approximately 2500 rpm and serum was taken and stored frozen at -20^{o}C until assay of Human Interleukin 21(IL-21). This work was carried out at Clinical Immunology Unit, Clinical Pathology Department, Mansoura Faculty of Medicine, Mansoura, Egypt.

Assay of Human Interleukin 21 (IL-21):

Assay of human interleukin 21 (IL-21) using enzyme-linked immunosorbent assay (ELISA) technique (NOVA, cat No. In-Hu2164, Bioneovan Co., Ltd, China), which assay the level of human interleukin 21 in serum.

Statistical analysis

Data were fed to the computer and analyzed using IBM SPSS software package version 22.0. Qualitative data were described using number and percent. Quantitative data were described using median and interquartile range for non-parametric data and mean, standard deviation (SD), and range (minimum and maximum) for parametric data after testing normality using Kolmogorov-Smirnov test. Significance of the obtained results was judged at the (0.05) level. Chi-Square test was used for comparison of qualitative data. Student t-test was used to compare parametric quantitative data. Mann-Whitney U test was

used to compare non-parametric quantitative data. Receiver Operating Characteristic (ROC) curve analysis: The diagnostic performance of a test, or the accuracy of a test to discriminate diseased cases from non-diseased cases was evaluated using Receiver Operating Characteristic (ROC) curve analysis. Sensitivity and specificity were detected from the curve and positive predictive value (PPV), negative predictive value (NPP) and accuracy were calculated through cross tabulation.

RESULTS

No significant difference was detected as regard age and sex between cases group and control group (Table 1).

Table (1): Demographic characteristics among studied groups

	Control n=40		Cases n=40		Test of significance
Age/years Range Mean ± SD	18-50 30.70±8.72		18-50 31.18±10.17		t=0.22 p=0.82
Sex	n	%	n	%	
Male	26	65.0	29	72.5	χ^2=0.52
Female	14	35.0	11	27.5	p=0.47

The median of disease duration among cases group was 3.5 months. Most of cases had negative family history (Table 2).

Table (2): Disease duration and family history distribution among studied cases

	Cases (n=40)	
	n=40	%
Duration of disease/ months Range (min-max) Median (interquartile range)	(0.25-24.0) 3.5 1.0-8.25	
Family history Negative Positive	33 7	82.5 17.5

Dermoscopic examination showed that each of exclamation mark sign and the black dots sign was positive in 29 patients. There were 27 patients with active AA (Table 3).

Table (3): Dermoscopic signs and disease activity among studied patients group

Cases (n=40)		
Dermoscopic sign	**N**	**%**
Exclamation mark sign Negative Positive	 11 29	 27.5 72.5
Black dots Negative Positive	 11 29	 27.5 72.5
Yellow dots Negative Positive	 33 7	 82.5 17.5
Dystrophies Negative Positive	 6 34	 15.0 85.0
Disease activity:		
Active Stable	27 13	67.5 32.5

IL-21 was significantly higher in cases when compared to control group (Table 4).

Table (4): Comparison of serum IL-21 between cases and control groups

	Control n=40	Cases n=40	Test of significance
Serum IL-21(Pg/ml) Mean ± SD	 12.14+2.83	 51.32+11.81	z=7.11 p<0.001*

The AUC-ROC of IL-21 was excellent and the cut of point between cases and control group was 22.22 (Table 5 and Figure 1).

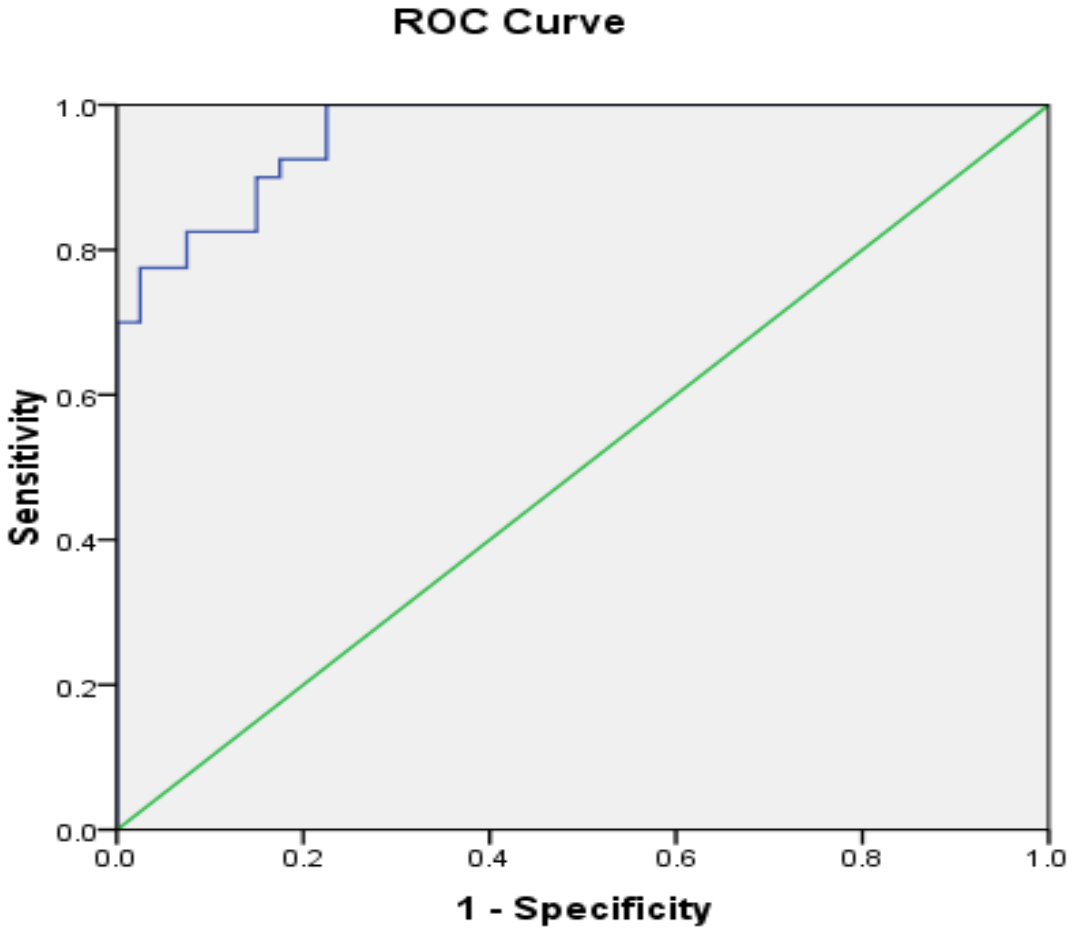

Figure (1): ROC curve for detecting validity of IL-21 in differentiating cases and control

Table (5): Validity of IL-21 in differentiating cases and control groups

	AUC (95% CI)	Cut off point	Sensitivity	Specificity	PPV	NPV	Accuracy
Serum IL-21 (pg/ml)	0.962 (0.93-0.99)	22.22	95.0%	77.5%	80.9%	93.9%	86.3%

AUC: Area under curve, PPV: Positive predictive value, NPV: Negative predictive value

No correlation was found between serum levels of IL-21 and each of age of patients and disease duration (Table 6).

Table (6): Correlation between serum level of IL-21 and each of age of patients and disease duration

IL-21 (Pg/ml)		
	r	p
Age/years	0.06	0.597
Duration/ months	-0.308	0.053

DISCUSSION

This is a case control study carried out on 40 patients diagnosed as alopecia areata with age range from 18 to 50 years old and 40 normal healthy subjects with matched age and sex. All patients were subjected to full history and general and dermatologic examination for scalp, body and nail. The scalp was examined for any hair shaft abnormalities, or signs of inflammation. The pattern of hair loss was determined. The severity of alopecia areata was assessed by SALT scoring. The activity of AA was determined by hair pull test and dermoscopic signs. We investigated the serum IL-21 level of patients, and healthy controls. We studied the correlation between IL-21 serum level and severity and activity of AA.

Bain *et al.* [12] found higher serum IL-21 levels in alopecia areata patients compared with healthy controls. Their results are in agreement with our results. As we found that the serum levels of IL-21 were significantly higher in patients with AA compared with the healthy controls. **Atwa *et al.*** [13] found non-significant differences in IL-21, IL-22 between patients with AA with different severity, so they are consistent with our data. In our study, there were no significant differences in IL-21 level with different SALT scores.

To our knowledge, no other studies were found to correlate between serum IL-21 and disease activity other than our study. In our study, we examined the relation between serum IL-21 and alopecia areata activity and severity. The activity of the disease was determined by clinical and dermoscopic signs. The patient with positive pull test, exclamation mark sign, and black dots sign was considered as active case. The patient, who had negative pull test and yellow dot sign by dermoscope, was considered as stable. In our study there were 27 patients with active AA, while 13 patients were with stable disease.

Our results denoted that there were a statistically significant differences in serum IL-21 in active cases compared with stable cases. The median (Interquartile range) of serum IL-21 level among patients with active alopecia areata was 67.13 (33.18-105.56) pg/ml, while it was 26.06 (22.17-38.64) pg/ml among patients with stable alopecia areata.

Also to our knowledge, no other studies were found to correlate between serum IL-21 and dermoscopic signs, but we studied the relation between serum IL-21 and different dermoscopic signs. The serum IL-21 levels showed significant elevation with positive exclamation mark and black dots signs, while it showed no significant elevation with yellow dots sign.

Receiver Operating Characteristic (ROC) curve analysis of IL-21 was conducted to evaluate the sensitivity and specificity of serum IL-21 as a diagnostic index for AA. The AUC-ROC of IL-21 was excellent (0.962); and the best cut off point for IL-21 was determined to be 22.22 pg/ml. It had good predictive value. Its accuracy was 86.3%.

The obtained data of serum IL-21 elevation in active alopecia areata suggest that IL-21 may contribute to disease induction and activity. This elevated levels in active disease could be explained by its action on suppressing effect of Treg cells on other T cells by elevating $CD4^+CD25^-$ T cells threshold for suppression by Treg.

Our results largely support the involvement of IL-21 cytokine in pathogenesis of alopecia areata. Higher serum levels of IL-21 in active cases support its role as predictor of disease activity. This study lays the ground work for understanding the pathogenesis of AA and suggests the role of IL-21 as potential therapeutic targets and as biomarkers of disease activity. Another study supports role of IL-21 in pathogenesis of alopecia areata, IL-2/IL-21containing genomic region on chromosome 4q27 is among the loci associated with AA [14].

The forementioned results are considered as a further evidence for role of TH17 in alopecia areata pathogenesis. This may be attributed to the role of IL-21 in activation and differentiation of TH17. To confirm TH17 role in AA**, Tembhre and Sharma** [15] **and El-Morsy *et al.*** [16] showed that IL-17A serum levels were significantly higher in AA patients than in healthy controls. **Tanemura *et al.*** [17] declared an evidence for TH17 role in AA. They found infiltration of $CD4^+$IL-$17A^+$ Th17 cells around hair follicles in 4 AA patients. Our results could explain the findings of **Shin *et al.*** [18] who revealed CD4+ CD25+ Treg cells dysfunction despite of their normal number in AA patients. To support the role of TH17 in AA, **Elela *et al.*** [19] evaluated the serum levels of IL-17, IL-22, Foxp3 and BAFF. Not only that but they also studied the tissue expression of Foxp3 and found that the serum levels of IL-17 were significantly higher in patients. They also denoted that Foxp3 immunestaining showed negativity in tissue of patients and controls.

CONCLUSION

The results of this study indicate that the serum IL-21 could be a promising marker in the diagnosis of alopecia areata, and also can be used as prognostic marker of its activity.

Financial support and sponsorship: Nil.
Conflict of interest: Nil.

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