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Original article

Serum Interleukin 18 and NLRP3 in Patients with Alopecia Areata

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Abstract

Background: Alopecia areata (AA) is a persistent hair follicle inflammatory condition with T lymphocyte invasion. Interleukin-18 (IL-18) and inflammatory proteins such as NOD-like receptor family pyrin domain containing 3 (NLRP3) have related to several inflammatory and autoimmune skin conditions. **Objectives:** To evaluate serum IL-18 and NLRP3 in patients with AA. **Materials and methods:** This case-control study comprised 50 patients with AA and 50 normal, healthy persons of comparable age and sex as a control group. Severity of AA was evaluated using the SALT score. ELISA kits were used to determine serum IL-18 and NLRP3 levels. **Results:** Serum IL-18 and NLRP3 levels were considerably greater in patients vs. controls (p= 0.01, <0.001, respectively). There was a substantial rise in IL-18 and NLRP3 levels as SALT score/disease severity increased (p= 0.03, 0.001). Patients with progressive course had notably higher levels of IL-18 and NLRP3 (p= 0.02, 0.01, respectively). There was a significant association between IL-18 and NLRP3 (p< 0.001). **Conclusion:** According to the findings, activation of the NLRP3 inflammasome and subsequent overproduction of IL18 could contribute to the pathophysiology of AA. Targeting this inflammatory process could be a useful treatment technique.

Keywords: Interleukin 18, NLRP3, Alopecia areata

Introduction

Alopecia areata (AA) is a non-scarring hair loss with an undefined cause [(1)]. The development of AA has been found to be greatly affected by innate immune responses, with clear links to viruses [(2)], mental strain [(3)], and oxidative stresses [(4)]. This is reinforced by cellular invasion of anagen hair follicles (HFs) with macrophages, natural killer (NK) cells, and lymphocytes [(5)].

NOD-like receptor family pyrin domain-containing 3 (NLRP3) belongs to pattern recognition receptors (PRRs). It has a crucial role in innate

immunity. Overactivation of PRRs frequently leads to unwanted inflammation and immunological responses. NLRP3 activation can trigger caspases, increase pro-inflammatory cytokines like IL-18, and lead to pyroptosis [6].

NLRP3 inflammasomes could have an integral part in the genesis of AA, according to various articles [7]. However, no comprehensive investigations on their role in the start of AA have been documented.

IL-18 stimulates IFN-γ release by NK cells, leading to increased Th1 multiplication and enhanced NK cell cytotoxicity. Because IL-18 has a harmful role in autoimmune disorders, cytokine production is an important aspect of the innate immune response [8]. As a result, we studied serum IL-18 and NLRP3 in AA patients.

Materials and methods

Study design and population:

This case-control study comprised fifty AA patients and fifty age and sex-matched healthy individuals as controls. They were recruited from the Dermatology Outpatient Clinic at Benha University Hospital during the period from January 2023 to June 2024.

Patients having AA of various severity levels were involved in the study. However, other kinds of alopecia, pregnant or breastfeeding women, patients with chronic inflammatory or autoimmune disorders, or those who had received immunosuppressive therapy in the three months prior to the examination, were not eligible to participate.

Methods:

All subjects underwent a complete history and a thorough general and dermatological examination. The severity of AA was determined employing Severity of Alopecia Tool (SALT) [9]. Enzyme-linked immune absorbent assay (ELISA) kits were used to estimate serum IL-18 (Catalog No. DL-IL18-Hu) and serum NLRP3 (Catalog No. DLR-NLRP3-Hu) supplied by DLdevelop, Wuxxi Donglin Sci&Tech Development Co., Ltd., China.

Ethical issues

The study had been authorized by the Benha Faculty of Medicine's human subjects research ethical committee (MS 20-9-2022). All participants provided informed written consent.

Statistical analysis

The data was calculated with SPSS Version 26 (IBM Corp., Armonk, New York). The distribution's normality has been verified by means of Kolmogorov-Smirnov test. Continuous data was presented as mean & standard deviation. Whereas categorical data was displayed as numbers & percent. Chi square and Kruskal-Wallis tests were used for analytical statistics. Correlations (Spearman/Pearson's technique) and Receiver operator characteristic (ROC) curve were also utilized. Values for P below 0.05 were regarded as significant.

Results

A total of 50 patients with AA were investigated, with a mean age of 30.38 $\pm$9.45 years. They consisted of 41 females (82%) and 9 males (18%). There was not a substantial distinction between patients and controls in terms of age or sex (p= 0.09, 0.3, respectively). Mean IL-18 and NLRP3

levels were significantly increased in cases versus controls (p=0.01, <0.001, respectively) (Table 1).

IL-18 and NLRP3 levels significantly correlated with SALT scores (p <0.001 for each). There was positive association between IL-18 and NLRP3 (P<0.001) (Table 2).

IL-18 and NLRP3 concentrations were considerably elevated in patients with exclamation marks (p=0.001, <0.0001, respectively), recurrent AA (P=0.003, 0.01, respectively), and progressive course (p=0.02, 0.01, respectively). There was a significant increase in both IL-18 and NLRP3 with increasing SALT score/disease severity (p<0.001) with highest levels in those with SALT score >50 (362.9±37.9 pg/ml, 8.44±1.2 ng/ml, respectively) (Table 3).

The ROC curves for IL-18 and NLRP3 demonstrated good identifying based on AUC values. Optimal cutoffs (212 pg/ml for 1L-18, 7.5 ng/ml for NLRP3) with corresponding sensitivity and specificity are also provided (Figures 1 and 2).

Table 1. Demographic and laboratory data of study groups

Characteristics		**Case (n=50)**		**Control (n=50)**		**T/X²**	***P***
Age (year) (mean ± SD)		30.38	9.45	33.40	7.97	1.7	0.09
Sex	Female	41	82	37	74	0.9	0.3
n. (%)	Male	9	18	13	26		
Family history of AA n. (%)	Yes	15	30	17	34	0.2	0.7
	No	35	70	33	66		
IL-18 (pg/ml)		251.73	89.63	208.41	79.78	2.6	0.01
NLRP3 (ng/ml)		7.99	1.96	6.10	2.65	4.1	<0.001
SALT score ((mean ± SD)		20.70±19.09					

Table 2. Correlation of serum IL-18 and NLRP3 with different variables

	Serum IL-18	
	r	*p*
Age	0.1	0.5
Duration of AA	0.3	0.08
SALT score	0.9	<0.001
NLRP3	0.42	<0.001
	serum NLRP3	
	r	*p*
Age	0.23	0.04
Duration off AA	0.2	0.09
SALT score	0.72	<0.001

Table 3. Comparison of IL-18 and NLRP3 levels among cases

SALT score	**<24 (n=37)**		**24-49 (n=7)**		**>50 (n=6)**		**F/KW**	***p***
	mean	±SD	mean	±SD	mean	±SD		
IL-18 (pg/ml)	244.4	81.1	319.6	177.3	326.9	37.9	3.9	0.03
NLRP3 (ng/ml)	5.2	2.2	8.4	3.8	8.44	1.2	13.7	<0.001
Exclamation marks			**Yes (n=12)**		**No (n=38)**		T	*P*
			mean	±SD	mean	±SD		
IL-18 (pg/ml)			278.88	98.39	211.02	40.31	3.4	0.001
NLRP3 (ng/ml)			8.65	1.72	5.99	1.64	4.8	<0.0001
Recurrence of AA			**Yes (n=16)**		**No (n=34)**		T	*P*
			mean	±SD	mean	±SD		
IL-18 (pg/ml)			299.70	98.56	224.80	66.49	3.2	0.003
NLRP3 (ng/ml)			8.18	2.78	6.06	2.63	2.6	0.01
Course of AA			**Progressive (n=28)**		**Stationary (n=22)**		T/U	*P*
			mean	±SD	mean	±SD		
IL-18 (pg/ml)			296.89	88.46	237.68	91.95	2.3	0.02
NLRP3(ng/ml)			7.50	2.55	5.58	2.76	2.5	0.01
Family history of AA			**Yes (n=15)**		**No (n=35)**		KW	*P*
			mean	±SD	mean	±SD		
IL-18 (pg/ml)			211.32	69.37	254.77	94.94	1.6	0.1
NLRP3 (ng/ml)			5.62	2.78	6.3	2.61	0.8	0.4

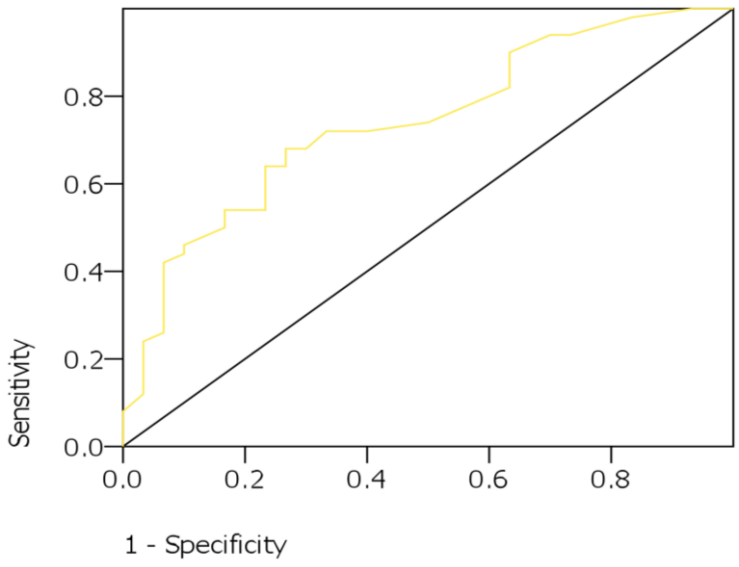

Figure 1. Roc curve for IL18 in prediction of AA

(Cutoff point=212pg/ml, AUC=0.74, Sensitivity= 72%, Specificity=67%)

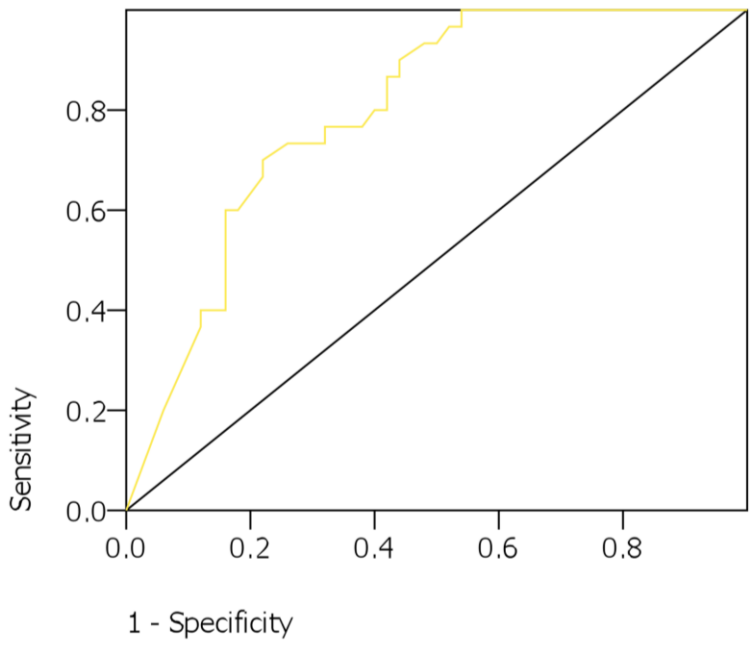

Figure 2. Roc curve for NLRP3 in prediction of AA

(Cutoff point 7.5ng/ml, AUC=0.8, Sensitivity= 77%, Specificity=68%)

Discussion

Alopecia areata results from loss of immunological privilege of HFs as well as the cytotoxic action of NK and CD8+ T cells. Studies suggest that AA is caused principally by deregulation of innate immunity [10]. We examined blood NLRP3 and compared it to IL-18 in relation to several variables, including severity of AA.

The present research found considerably higher mean serum NLRP3 levels in AA patients when compared with controls, with a positive connection with SALT score and the progressive course. This is consistent with the strong expression of NLRP3 components in outer root sheath of HFs in lesions of AA [7, 11]. Moreover, Lee and Bae [12] found a link between the NLRP3 gene polymorphism and autoimmune conditions.

NLRP3 inflammasome participates in the pathogenesis of autoimmune disorders like lupus erythematous [13] and rheumatoid arthritis [14, 15], principally via regulating downstream cytokines implicated in their start and progression [16].

The present findings demonstrated a substantial connection between NLRP3 and IL-18. This could be explained by the activation of NLRP3, which activates pro-IL-18. Active IL-18 promotes chemotaxis and cellular proliferation, leading to adaptive immunological reactions [17]. In the same context, reduced NLRP3 expression decreases IL-18 production, restricting development and

responsiveness of Th1 and Th17 cells [18].

The current study found that serum IL-18 concentration was considerably higher in AA patients vs. controls and linked favorably with SALT score. This is consistent with Gilhar et al. [19] and El-Gayyar et al. [20]. IL-18 has been identified as an encouraging factor for IFN-γ-, which is the primary inducer of AA [21].

The imbalance in cytokine production, with a relative increase in pro-inflammatory cytokines like IL-18 vs. anti-inflammatory ones, may enhance persistence of AA lesions, as demonstrated in human scalp biopsies [22]. In contrast to the current study, Lee et al. [23] did not observe any noteworthy difference in IL-18 measurements across diverse grades of AA.

ROC curve analysis of IL18 and NLRP3 demonstrated good discrimination between AA cases and controls based on AUC values. Optimal cutoffs with corresponding sensitivity and specificity were also shown.

Limitations:

A bigger sample size and multi-center design of the study should be considered in the future. Investigation and linkage of IL-18 and NLRP3 tissue levels with serum levels is recommended.

Conclusion:

The data imply that the NLRP3 inflammasome is involved in the pathophysiology of AA via influencing downstream IL-18 expression. Both NLRP3 and IL-18 are linked to the severity of AA. Targeting this inflammatory process could be a useful treatment technique.

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