Clinical characteristic, laboratory biomarkers, treatment regimen and psychiatry problems predictors of outcomes of alopecia areata: a prospective study

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Background
Alopecia areata (AA) has multiple aetiology such as genetic and environmental triggers.
Aims
To assess the recovery rate of AA and examine the associated psychiatric problems. Additionally, the relationship between clinical, psychiatric, and laboratory biomarkers and alopecia outcomes were investigated, along with potential risk factors that could aid in treating alopecia.
Patients and methods
A prospective cohort research included 42 AA patients and 45 healthy controls. Group A (active disease), group B (inactive disease), and group C (healthy control) were based on illness outcomes after 3 months of treatment. The Severity of Alopecia Tool (SALT), treatment regimens, laboratory investigation Interleukins 19 and 33 (IL-19 and IL-33), Symptom Checklist 90, and post-traumatic stress disorder Checklist for DSM-5 (PCL-5) were evaluated.
Results
After 3 months of therapy, the incidence of inactive AA was found to be 57.14%. Being females with family history of dermatitis were highly related with active illness, while smoking and unmarried patients were associated with inactive disease. After 3 months of treatment, active illness had the highest mean IL-33 and IL-19 levels.
Conclusion
The active disease group exhibited the highest mean IL-33 and IL-19 levels at baseline following three months of treatment. Our patients had 7.1% somatization, 7.1% obsessive-compulsive symptoms, 4.8% depression, 4.8% anxiety, 15.9% anger-hostility, 35.7% phobic-anxiety, 26.2% paranoid ideation, 4.8% psychoticism, and 61.9% post-traumatic stress disorder. AA outcomes were linked to females, a family history of dermatological disorders, smoking, being single, and higher mean IL-33 and IL-19 levels. Psychosis was highly linked with active AA. Only khellin and Ultraviolet A improved AA results.

Keywords:
alopecia areata, clinical evaluation and treatment, immunohematology, psych dermatology

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List of Abbreviations: AA, alopecia areata; IL33and IL19, Interleukins 19 and 33; KUVA, khellin and Ultraviolet A; PTSD, post-traumatic stress disorder; SALT score, The Severity of Alopecia Tool; TOP, topical steroid application.

Introduction

Alopecia areata (AA) is a nonscarring hair disorder that affects ~0.5–2% of the global population, and its specific cause is still unknown. Multiple factors such as genetic inheritance, environmental triggers, impaired hair growth, and altered inflammatory and immunological systems contribute to the development of this condition [1].

Imbalances in several systemic helper T cells (Th1), Th2, and Th17 cytokines have been observed in patients with AA [2]. Additionally, proinflammatory cytokines belonging to the Interleukins (IL-1) family have been linked to the etiology of this disease [3]. For example, IL-33, an IL-1 family cytokine produced by both immune and nonimmune cells [4], interacts with the ST2 receptor, which exists in two forms: transmembrane (ST2L) and soluble (sST2) [5]. The transmembrane form is expressed in various cell types, including Th2 and Th1 cells, CD8+ cells, Tregs, and mast cells, and it plays a role in initiating immune-mediated inflammatory responses [6]. IL-19, another

biomarker belonging to the IL-10 subfamily [7], possesses unique characteristics such as target cell modulation and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB)-independent actions. It is also induced by epithelial cells in response to stimulation [8]. However, IL-19 does not promote immunoglobulin synthesis [9]. Hair follicle-specific immunoglobulin neutralizer concentrations are higher in the peripheral blood of patients with AA [10]. Recent advancements in understanding the immunological mechanisms involved have led to the developing of new therapeutic options, although the response rates vary [1].

On the other hand, it has been suggested that AA could be a psychosomatic disorder triggered by stressful life events. Therefore, it has been proposed to classify AA as either a primary dermatological condition with associated mental health conditions or a primary mental health disorder with dermatological complications [11]. Most quantitative research has predominantly centered on anxiety and depression concerning AA. Okhovat *et al.*'s meta-analysis, encompassing eight studies, disclosed that adults with AA are 2.50 times more likely to experience anxiety [12]. Other studies have further suggested that adults with AA exhibit a heightened likelihood of being diagnosed with an anxiety disorder compared with healthy controls [13]. When contrasting the level of anxiety symptoms in individuals with AA to those with other dermatological diagnoses, mixed results have been reported [14]. Regarding depression, the meta-analysis revealed that adults with AA are 2.71 times more likely to experience depression [12]. Similar to anxiety, the comparison of depression levels between individuals with AA and those with other dermatological diagnoses remains unclear [15,16]. Few studies reported other psychiatric problems for example, previous studies have reported present of obsessive-compulsive disorder in individuals with AA [17]. Furthermore, descriptive studies have indicated that people with AA experience significant emotional distress, including sadness, insecurity, inadequacy, anxiety, suicidal thoughts, and self-consciousness [18]. Moreover, these studies have shown that individuals with AA face difficulties in daily activities such as sports or social events due to fear of judgment based on their appearance [19]. These findings suggest a shared etiology between AA and mental health disorders, and some theories emphasize the role of neuroendocrine immunology in this relationship [20].

Therefore, this study aimed to assess the recovery rate of AA and explore the associated psychiatric problems. Additionally, the study aimed to examine the relationships between clinical, psychiatric, and laboratory biomarkers and the outcomes of alopecia areata and investigate potential risk factors that could aid in treating this condition.

Patients and methods

Participants and study setting

A prospective cohort study enrolled 42 individuals diagnosed with AA from the Dermatology, Andrology, and Venerology Department at [blinded for peer-review] outpatient clinic. The study was initiated on January 1, 2022, and concluded on February 1, 2023. Initially, 45 patients with AA were enrolled; however, two participants did not complete the study by not returning for follow-up. The study also included 45 healthy participants as a control group.

Individuals with other hair diseases, concurrent infectious, inflammatory, or autoimmune cutaneous or systemic conditions, and pregnant and lactating females were excluded from the study. Participants were categorized into three groups based on their disease outcomes after three months of treatment: group A: active disease, group B: inactive disease, and group C: healthy control.

We determined that the inactive AA group was inactive based on a comprehensive analysis. A systematic review [21] highlighted the five most distinctive trichoscopic findings in AA, namely yellow dots, black dots, broken hairs, short vellus hairs, and tapering hairs. Among these, yellow dots and short vellus hairs were identified as the most sensitive indicators for AA, whereas black dots and tapering hairs were deemed the most specific. Moreover, trichoscopy emerged as a valuable tool for monitoring treatment response in AA cases. Positive responses to treatment were characterized by an increase in short vellus hairs. Conversely, tapering hairs, broken hairs, and black dots exhibited a reduction in treated cases. Notably, yellow dots demonstrated the least responsiveness to the applied treatments. This comprehensive trichoscopic analysis allowed us to discern the inactive status of the AA group under consideration [21].

Procedure

Sociodemographic and clinical data

A comprehensive medical history and physical examination were conducted for all patients. The scalp was examined to determine the extent, location, and size of alopecia patches, while the

entire body was thoroughly examined to identify any alopecia patches in hairy areas. Nail examinations were also performed to assess nail involvement.

The Severity of Alopecia Tool (SALT) was utilized to evaluate the severity of AA and the clinical response, following the AA Investigational Guidelines. The SALT score is calculated by determining the percentage of hair loss in four areas of the scalp (vertex: 40%, right profile: 18%, left profile: 18%, and posterior: 24%) and then summing up the individual scores. A decrease in the SALT score indicates hair regrowth, with a score of 0 indicating complete regrowth [22].

Treatment regimens

Two types of treatment were administered to the patients. The first treatment involved a three-month course of topical steroid application (TOP) twice daily. The second treatment involved the administration of KUVA (khellin and Ultraviolet A) twice a week for three months.

Laboratory investigation: Interleukins 19 and 33 (ILs)

A serum separator tube collected a 2 ml venous blood sample from each participant. After clotting for 30 min at room temperature, the samples were centrifuged at 1000×g for 15 min. The serum samples were separated and stored at −20°C. Upon testing, the samples were thawed and subjected to a Magnetic Luminex Assay (Multiplexed sandwich ELISA) using the Labscan three-dimensional system, following the manufacturer's protocol (Luminex Discovery Assay: Human premixed multi-Analyte Kit, USA, Catalogue No. LXSAHM-02, Lot No. 145450).

The magnetic microparticles used in the assay were pre-coated with antibodies specific to interleukins bound to the interleukins in the samples and standards. After removing any unbound substances through washing, a biotinylated antibody cocktail specific to the interleukins was added to each well. Following another wash to remove the unbound biotinylated antibody, streptavidin-phycoerythrin conjugate (Streptavidin-PE) was introduced to bind to the biotinylated antibody. After a final wash to remove unattached Streptavidin-PE, the microparticles were resuspended in buffer and analyzed using the Luminex MAGPIX Analyzer. The analyzer employed a magnet to hold the superparamagnetic microparticles in a monolayer, and two spectrally different Light Emitting Diodes (LEDs) illuminated the beads. The first LED identified the specific interleukins being detected, while the second LED measured the magnitude of the PE-derived signal, which was proportional to the bound interleukins. An imaging CCD camera was used to capture the images of each well. The results were expressed in pictograms per millilitre (pg/ml).

Psychometric scales: The study employed two psychometric scales: the Symptom Checklist 90 (SCL-90) [23] and PTSD Checklist for Diagnostic and Statistical Manual of Mental Disorders(DSM)-5 (PCL-5) [24]. The SCL-90 is a 90-item psychological assessment questionnaire that evaluates the degree of disturbance caused by each item on a scale of 0–4. It consists of nine subscales. The PCL-5 is a 20-item self-report measure based on the DSM-5 criteria for diagnosing post-traumatic stress disorder (PTSD). A cut-off score of 33 or higher on the PCL-5 indicates PTSD [25].

After 3 months, patients were evaluated by their disease outcomes and categorized into two groups.

Statistical analysis

The statistical analysis was conducted using SPSS version 26 (IBM, Armonk, NY, USA). The mean and standard deviation were calculated for continuous data, while the frequency (percent) was computed for nominal data. The independent *t*-test and Mann–Whitney test were used to compare means between the two groups, and the χ^2 test was employed for nominal data comparison among the three study groups. The ANOVA or Kruskal-Wallis test was used to compare mean data among the three study groups. Univariate and multivariate linear models were performed to identify risk factors for active alopecia. A P value less than 0.05 was considered statistically significant.

Results

Sociodemographic and clinical data

In this study, we recruited 42 patients with AA and categorized them into two groups based on their disease outcomes after three months of treatment. The incidence of inactive AA was 57.14% after three months of therapy. Therefore, we divided the groups: group A, active disease (N=18), and group B, inactive disease (N=24). Additionally, we included 45 healthy individuals in group C, the control group. Statistical analysis revealed significant differences between groups A and B regarding sex, smoking status, family history of dermatological conditions, treatment regimens, and SALT score (Table 1).

Group A had a higher proportion of females (72.2%) than group B (33.3%). Group B received a higher proportion of KUVA therapy (75%) and had more smokers (41.7%) than group A. On the other hand, group A had higher SALT scores and a higher prevalence of family history of dermatological diseases compared with group B (15.33±5.477 vs. 12 ±6.33, 38.9% vs. 12.5%, respectively).

When comparing the three groups, statistically significant differences were found in sex, marital status, family history of dermatological diseases, and IL33 and IL19 measures. Group A had a higher proportion of females (72.2%) compared with the other groups, while married participants were less frequent in group A compared with the other groups. The control group had the lowest mean levels of IL33 and IL19 compared with the other groups.

Results of SCL-90 scale and PCL-5

Among the case groups, group A had a higher frequency of psychosis (11.1%), while group B had no psychotic symptoms. Conversely, there was a statistically significant difference between the control and case groups regarding obsession, depression, anxiety, and phobic anxiety. The case group had a higher percentage of abnormal responses in these domains than the control healthy group. Statistically significant differences were also observed among the three groups regarding anxiety and phobic anxiety. Group A and B had a higher prevalence of borderline anxiety (more than one-third of participants) than the controls (8.9%). Additionally, approximately one-third of group A and group B participants had abnormal responses of phobic anxiety compared with the controls (11.1%) (See Table 2).

Relational studies

The univariate regression analysis for activating alopecia with other parameters is presented in Table 3. In terms of clinical variables, being female (P=0.016) and receiving TOP (P=0.001) were associated with increased susceptibility to alopecia. Conversely, receiving KUVA (P=0.001) and being a smoker (P=0.042) were associated with a decreased risk of alopecia. In the multivariate regression analysis, only using KUVA (P=0.005) was associated with a decreased risk of active alopecia (See Table 4).

Discussion

This study aimed to evaluate the recovery rate of AA and its association with psychiatric problems. We also investigated the potential link between clinical, psychological, and laboratory indicators and alopecia outcomes and examined potential risk factors for the treatment of alopecia. The incidence of inactive AA was 57.14% after three months of therapy. Patients with AA were enrolled and divided into two groups based on their disease outcomes after three months of treatment: group A (active disease) (N=18) and group B (inactive disease) (N=24). Additionally, 45 healthy individuals were included in group C, the control group.

Biomarkers in clinical practice

The current study revealed a strong association between females and a family history of dermatitis in the active group after 3 months of treatment. Conversely, smoking and being unmarried were significantly associated with the inactive group.

The unexpected characteristics of AA have also been noted as a source of worry in particular [18] with women than men [26]. Stress and oxidative stress have been suggested as potential factors in the development of AA. Previous research has indicated that individuals with AA who experience higher levels of psychosocial stress exhibit a specific polymorphism in their adrenocorticotropin receptor, leading to an inadequate hormonal response to stress [27]. Another study has identified a direct relationship between stress and AA, as cellular membranes exposed to reactive oxygen species produce by-products that can impair cellular function. Significant levels of these metabolites have been detected in AA patients' plasma, red blood cells, and scalps [28].

Smoking increases pro-inflammatory and decreases anti-inflammatory cytokines [29]. Dai and colleagues found that current smokers were more likely to acquire AA than never-smokers, and the risk rose with the number of smoking years and cigarettes smoked [30]. Many hypotheses have been offered about how cigarette smoking affects hair follicles. The precise mechanism of cigarette smoking affects hair follicles remains unknown, although various theories have been proposed [31].

Smoking affects humoral and cell-mediated immune responses and has been connected to several disease disorders. Smoking releases cytokines such as TNF-, IL-1, and IL-6, which may cause an inflammatory reaction around the hair follicle [30]. Hair follicles usually have 'immune privilege', protecting them from systemic immunological attacks [30,31]. Smoking

Table 1 Sociodemographic, clinical and laboratory data among the studied groups

Variables	Group A (*N*=18)	Group B (*N*=24)	Total cases (*N*=42)	X^2 or T value 1	*P*1-value	Group C (*N*=45)	X^2 or T value 2	*P*2-value of group C versus total cases	X^2 or T value 3	*P*3-value of 3 groups
Age	21.89±10.55	25.21±7.18	25.2±7.45	–1.213	0.232	26.5±5.69	1.251	0.215	2.559	0.083
	21.5 (12.5–31)	26 (17.25–31.5)	26 (20–31)	–1.082	0.279	27 (22.5–30)	–0.689	0.491	2.48	0.289
Sex										
Male	5 (27.8%)	16 (66.7%)	21 (50%)	6.222	0.013*	8 (17.8%)	10.15	0.001*	17.15	0.0001*
Female	13 (72.2%)	8 (33.3%)	21 (50%)			37 (82.2%)				
Marital status										
Single	11 (61.1%)	11 (45.8%)	22 (52.4%)	0.962	0.327	11 (24.4%)	7.201	0.007*	8.221	0.016*
Married	7 (38.9%)	13 (54.2%)	20 (47.6%)			34 (75.6%)				
Smoking	2 (11.1%)	10 (41.7%)	12 (28.6%)	4.706	0.03*	7 (15.6%)	2.156	0.142	7.782	0.02*
History of other medical conditions	5 (27.7%)	8 (33.3%)	13 (31%)	0.149	0.7	8 (17.8%)	2.059	0.151	2.233	0.327
Family history of dermatological disease	7 (38.9%)	3 (12.5%)	10 (23.8%)	3.948	0.07*	0	12.106	0.0001*	19.147	0.0001*
Duration of illness (days)	870.33±1707.57	384.38±723.29	592.64±1249.68	1.256	0.216	–	–	–	–	–
	255 (112.5–720)	225 (90–322)	225 (90–360)	–0.866	0.386					
Nail involvement#	4 (22.2%)	5 (20.8%)	9 (21.4%)	0.012	0.914	–	–	–	–	–
Previous attack#	9 (50%)	7 (29.2%)	16 (38.09%)	1.893	0.169	–	–	–	–	–
KUVA	4 (22.2%)	18 (75%)	22 (52.38%)	11.486	0.001*	–	–	–	–	–
TOP	14 (77.8%)	6 (25%)	20 (47.62%)			–	–	–	–	–
SALT score	15.33±5.477	12±6.33	13.43±6.14	1.787	0.082		–	–	–	–
	14 (10–20)	10 (8–14.75)	11.5 (8.75–18.25)	–2.256	0.024*					
IL33	8.88±2.14	8.19±2.15	7.14±2.4	1.026	0.311	5.89±1.909	–7.527	0.000*	18.44	0.000*
	8.8 (7.31–10.19)	8.61 (6.3–9.79)	7.014 (5.8–9.008)	–1.057	0.291	5.8 (4.98–7.41)	–5.604	0.000*	25.869	0.000*
IL19	9657.5±19126.39	4603.23±7729.66	4200.74±9948.026	1.177	0.246	1803.3755 ±1646.104	–2.985	0.004*	4.348	0.016*
	3307.34 (1466.74–5467.86)	2306.62 (1466.74–4308.07)	1890.75 (1031.68–3915.71)	–0.663	0.507	1890.75 (281.23–2716.08)	–3.395	0.001*	11.979	0.003*

Group A, Active disease; Group B, inactive disease; Group C, healthy control; IL33and IL19, Interleukins 19 and 33; KUVA, khellin and Ultraviolet A; SALT score, The Severity of Alopecia Tool; TOP, topical steroid application. *P*1: active group vs inactive group; *P*2: case group versus control group, *P*3: active versus inactive versus control group. *Significant *P* value.

Table 2 Results of Symptom Checklist 90 and PCL-5 among the studied groups

Variables	Group A (*N*=18)	Group B (*N*=24)	Total cases (*N*=42)	X^2 or T value 1	*P*1-value	Group C (*N*=45)	X^2 or T value 2	*P2*-value of group C versus total cases	X^2 or T value 3	*P*3-value of 3 groups
Somatization										
Normal	8 (44.4%)	9 (37.5%)	17 (40.5%)	0.574	0.751	19 (42.2%)	0.295	0.863	1.653	0.799
Borderline	8 (44.4%)	14 (58.3%)	22 (52.4%)			24 (53.3%)				
Abnormal	2 (11.1%)	1 (4.2%)	3 (7.1%)			2 (4.4%)				
Obsessive-compulsive										
Normal	10 (55.6%)	14 (58.3%)	24 (57.1%)	0.567	0.753	38 (84.4%)	8.268	0.016*	9.22	0.056
Borderline	6 (33.3%)	9 (37.5%)	15 (35.7%)			5 (11.1%)				
Abnormal	2 (11.1%)	1 (4.2%)	3 (7.1%)			2 (4.4%)				
Interpersonal sensibility										
Normal	11 (61.1%)	14 (58.3%)	25 (59.5%)	0.099	0.5	33 (73.3%)	1.864	0.183	1.900	0.387
Borderline	7 (38.9%)	10 (41.7%)	17 (40.5%)			12 (26.7%)				
Abnormal	0	0	0			0				
Depression										
Normal	12 (66.7%)	14 (58.3%)	26 (61.9%)	2.154	0.341	39 (86.7%)	7.101	0.029*	7.700	0.103
Borderline	5 (27.8%)	9 (37.5%)	14 (33.3%)			5 (11.1%)				
Abnormal	1 (5.6%)	1 (4.2%)	2 (4.8%)			1 (2.2%)				
Anxiety										
Normal	11 (61.1%)	12 (50%)	23 (54.8%)	2.573	0.276	40 (88.9%)	12.880	0.002*	13.779	0.008*
Borderline	6 (33.3%)	11 (45.8%)	17 (40.5%)			4 (8.9%)				
Abnormal	1 (5.6%)	1 (4.2%)	2 (4.8%)			1 (2.2%)				
Anger-hostility										
Normal	15 (83.3%)	21 (87.5%)	36 (85.7%)	0.667	0.717	31 (68.9%)	7.529	0.023*	7.994	0.092
Borderline	2 (11.1%)	1 (4.2%)	3 (7.1%)			13 (28.9%)				
Abnormal	1 (5.6%)	2 (8.3%)	3 (7.1%)			1 (2.2%)				
Phobic anxiety										
Normal	9 (50%)	11 (45.8%)	20 (47.6%)	3.610	0.165	16 (35.6%)	14.681	0.001*	15.135	0.004*
Borderline	2 (11.1%)	5 (20.8%)	7 (16.7%)			24 (53.3%)				
Abnormal	7 (38.9%)	8 (33.3%)	15 (35.7%)			5 (11.1%)				
Paranoid ideation										
Normal	8 (44.4%)	11 (45.8%)	19 (45.2%)	0.477	0.788	24 (53.3%)	4.612	0.10	4.668	0.323
Borderline	5 (27.8%)	7 (29.2%)	12 (28.6%)			17 (37.8%)				
Abnormal	5 (27.8%)	6 (25%)	11 (26.2%)			4 (8.9%)				
Psychosis										
Normal	15 (83.3%)	19 (79.2%)	32 (81%)	8.471	0.014*	36 (80%)	2.557	0.278	9.49	0.05
Borderline	1 (5.6%)	5 (20.8%)	6 (14.3%)			9 (20%)				
Abnormal	2 (11.1%)	0	2 (4.8%)			0				
PCL-5										
Not PTSD	6 (33.3%)	10 (41.7%)	16 (38.1%)	0.303	0.75	24 (53.3%)	2.031	0.198	2.318	0.314
Probably PTSD	12 (66.7%)	14 (58.3%)	26 (61.9%)			21 (46.7%)				

Group A, Active disease; Group B, inactive disease; Group C, healthy control; PCL-5, PTSD Checklist for DSM-5; SCL-90, Symptom Checklist 90. *P*1: active group vs inactive group, *P*2: case group versus control group, *P*3: active versus inactive versus control group. *Significant *P* value.

Table 3 Univariate regression for activate Alopecia with other parameters

						95.0% confidence interval for B	
	B	S.E.	Wald	Significance	Exp (B)	Lower	Upper
Age	0.045	0.037	1.454	0.228	1.046	0.973	1.124
Female sex	–1.649	0.681	5.853	0.016*	0.192	0.051	0.731
Married	0.619	0.634	0.954	0.329	1.857	0.536	6.431
Smoking	1.743	0.857	4.139	0.042*	5.714	1.066	30.633
History of other medical conditions	0.262	0.681	0.148	0.700	1.300	0.342	4.943
Family history of dermatological disease	–1.494	0.784	3.631	0.057	0.224	0.048	1.044
Nail involvement	–0.082	0.758	0.012	0.914	0.921	0.209	4.066
Previous attack	–0.887	0.651	1.857	0.173	0.412	0.115	1.475
KUVA	2.351	0.737	10.170	0.001*	10.500	2.475	44.545
TOP	–2.351	0.737	10.170	0.001*	0.095	0.022	.404
SALT score	–0.094	0.055	2.898	0.089	0.910	0.817	1.014
IL33	–0.155	0.151	1.053	0.305	0.856	0.636	1.152
IL19	–0.00003	0.000028	1.152	0.283	1.000	1.000	1.000
Somatization	–1.056	1.267	0.694	0.405	0.348	0.029	4.169
Obsessive-compulsive	–1.056	1.267	0.694	0.405	0.348	0.029	4.169
Interpersonal sensibility	–1.056	1.267	0.694	0.405	0.348		
Depression	–0.302	1.450	0.043	0.835	0.739	0.043	12.674
Anxiety	–0.302	1.450	0.043	0.835	0.739	0.043	12.674
Anger-hostility	0.435	1.267	0.118	0.731	1.545	0.129	18.501
Phobic anxiety	–0.241	0.649	0.138	0.710	0.786	0.220	2.804
Paranoid ideation	–0.143	0.707	0.041	0.839	0.867	0.217	3.461
Probably PTSD	–0.357	0.649	0.302	0.583	0.700	0.196	2.498

Psychosis is not included due to multicollinearity. *Significant *P* value.

Table 4 Multivariate regression for activate alopecia with other parameters

						95.0% confidence interval for B	
	B	S.E.	Wald	Significance	Exp(B)	Lower	Upper
Female sex	–0.785	0.965	0.662	0.416	0.456	0.069	3.022
Smoking	1.154	1.185	0.949	0.330	3.172	0.311	32.350
KUVA	2.237	0.788	8.053	0.005*	9.363	1.998	43.889

*Significant *P* value.

accumulates free radicals in hair follicles and may disrupt immunological privilege and cause AA [30].

Other hypothesis suggested that smoking has paradoxical effects in psychiatry, as it can reduce mental stress while increasing physiological alertness. Previous studies utilizing event-related potential have demonstrated that smoking can diminish arousal and potentially reduce stress in smokers [32]. Additionally, nicotine, through its interaction with nicotinic acetylcholine receptors (nAChRs), can modulate pathways involved in stress response, anxiety, and depression. Nicotine's effects on emotionality are complex due to the broad expression of nAChRs in the brain, the multitude of nAChR subtypes, and nicotine's ability to activate and desensitize these receptors. The activity of stress-related systems, including stress hormone pathways and neurotransmitter release, can be influenced by nAChR activation. Nicotine can act as an anxiolytic and an antidepressant, but long-term use may lead to nicotine adaptations and increased anxiety and depression during withdrawal [33]. Furthermore, nicotine use is more prevalent among men, which may explain the higher proportion of smokers in the inactive group of AA patients.

The severity, comorbid problems, and family history of AA patients suggest genetic factors. Two clinical subgroups of AA, one with more severe manifestations and higher incidence in patients with affected family members, have also been supported [34].

Laboratory biomarkers

Following 3 months of treatment, the active group exhibited the highest mean IL-33 and IL-19 levels at

baseline. This finding aligns with Eid *et al.*'s research [35], which demonstrated significantly higher IL-33 levels in AA patients than in controls. Furthermore, IL-33 levels were positively correlated with the severity of clinical disease and the severity of alopecia measured by the SALT score. The activation of the IL-33 receptor stimulates the immune system, releasing proinflammatory cytokines and chemokines from immune cells upon contact with IL-33 [36]. AA hair follicle cells and dendritic cells are believed to display increased action of MHC class I and II, presenting autoantigens to CD8+ T cells, CD4+ T cells, and natural killer (NK) cells [37]. These hair follicle cells also produce cytokines and chemokines that target CD4+ T cells and NK cells [38].

Previous studies have demonstrated that the combination of IL-33 and IL-12 treatment ex vivo induces high levels of IFN-γ in iNKT and human NK cells [39]. IL-33 and IL-12 have also enhanced IFN production and CD8+ T-cell effector activity [40]. Interferon (IFN) plays a significant role in recruiting follicular leukocytes [41], and its binding to IFN receptors triggers the release of cytotoxic granzymes like Granzyme B. The accumulation of cytotoxic granules and IFN leads to the destruction of hair follicle cells and the disruption of the hair growth cycle [42].

The administration of exogenous IL-33 causes the expansion and activation of innate immune cells such as type 2 innate lymphoid cells (ILC2), mast cells, and basophils. These cells secrete type 2 cytokines (IL-4, IL-5, IL-9, and IL-13), chemokines, and proinflammatory mediators, including proteases, histamine, eicosanoids, and IL-6 [4]. The significant relationship between IL-33 and Th1 and Th2 type cytokines may explain the increased IL-33 levels and its association with illness severity and chronicity in this study.

Moreover, the patient groups exhibited significantly higher levels of IL-19 than the control groups, and the active group had higher levels of IL-19 than the inactive group. This finding is consistent with the research conducted by Ebrahim *et al.* (2020), which reported higher serum IL-19 levels in all AA patients compared with the control group In patients with alopecia areata, CD4+, and Th17 cells were found to infiltrate the dermis, particularly around hair follicles [43]. A Th17-related autoimmune mechanism has been proposed as a potential cause of AA [44].

Th17 cells activated by IL-23 generate various cytokines, including tumor necrosis factor (TNF), INF, IL6, IL17A, IL17F, IL21, and IL22 [45]. IL-17A induces inflammatory cytokines and chemokines that attract neutrophils, dendritic cells (DCs), and T lymphocytes [46]. IL-19 has been shown to enhance the activity of IL-17A, potentially through signal transduction [47].

The restoration of various cytokine levels in the blood, comparable with healthy controls, after effective treatment for hair loss, indicates a connection between systemic cytokine dysregulation and the prevalence of AA [48].

Relation between alopecia and psychiatry problems

The present study identified various psychiatric symptoms among AA patients. The percentage of somatization was found to be 7.1%, obsessive-compulsive symptoms 7.1%, depression 4.8%, anxiety 4.8%, anger-hostility 15.9%, phobic-anxiety 35.7%, paranoid ideation 26.2%, psychoticism 4.8%, and PTSD 61.9%. Compared with control groups, the active AA group exhibited a significantly higher percentage of psychosis at 11.1%. Additionally, the cases group had higher rates of obsession, depression, anxiety, and phobic anxiety than the control healthy group.

The frequency of depression in AA patients ranged from 2.9% [20] to 3.98% [49]. There is a growing belief that depression may precede the onset of AA. Stressful life experiences were reported by 25% of AA patients before or during the condition, and other factors such as family issues, employment difficulties, poor self-confidence, social difficulties, worries and grief were also highlighted [50]. Antidepressant imipramine decreases depression and increases hair regeneration, suggesting that AA may work similarly [51].

In 1993, Kubota and colleagues reported that atypical antipsychotic Zotepine caused AA in a schizophrenic patient. After stopping Zotepine [52]. AA resolved. Schizophrenia and AA may have different pathophysiology, thus more research is needed to find new therapies. Schizophrenia was considerably lower in AA patients than matched controls in a Taiwanese case-control study [20].

Prevalence rates of anxiety among AA patients have been reported in prior research, ranging from 3.24% [53] to 13.70% [54]. The prevalence of specific anxiety disorders among AA patients was 23% for specific phobia [55]. In addition, obsessive-compulsive disorder was found to be the most prevalent

coexisting anxiety disorder among AA patients, with a rate of 35.7% [17].

Possible risk factors in recovery process of AA

Potential risk factors for AA recovery were investigated. A univariate regression model revealed that TOP treatment in female participants increased their susceptibility to AA activation. Conversely, using KUVA and smoking were associated with a lower risk of activated AA. However, in a multivariate regression model, only KUVA treatment significantly reduced the likelihood of active AA. The efficacy of KUVA therapy in AA treatment was examined by Meguid and colleagues that reported KUVA therapy outperforms NB-UVB therapy in treating AA that is resistant to other treatment approaches [56].

This study had several limitations. Firstly, the small sample size resulted from difficulties in recruitment. Secondly, repeated measurements of laboratory biomarkers were necessary to assess the changes before and after three months of treatment.

Conclusion

After three months of therapy, the prevalence of inactive AA was 57.14%. Several factors, including being female, having a family history of dermatological conditions, smoking, being single, and having higher levels of IL-33 and IL-19, were associated with the outcome of AA. Furthermore, psychiatric symptoms were observed in our patients, including somatization was (7.1%), obsessive-compulsive was (7.1%), depression was (4.8%), anxiety was (4.8%), anger-hostility was (15.9%), phobic-anxiety was (35.7%), paranoid ideation was (26.2%), psychoticism was (4.8%), and PTSD was (61.9%), with a particularly high percentage of psychosis in cases of active AA. Among the identified factors, KUVA treatment was found to have a potentially favorable effect on the outcome of AA.

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