

Evaluation of CD4+, CD39+, FOXP3+Tregulatory cells in Iraqi Alopecia areata patients by ELISA

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ABSTRACT

An autoimmune condition known as *Alopecia areata* (AA) affects the hair follicles and causes patches of noncicatricial hair loss in any area of the body with hair. The purpose of this research was to assess the presence of CD4+, CD39+, FOXP3+ Treg cells in patients with *Alopecia areata* in Iraq. This investigation was conducted at a private dermatological clinic. 45 clinically diagnosed patients and 45 healthy controls from various regions of Iraq were included in the case study. Their ages ranged from 5 to 59. Using ELISA, CD4+, CD39+, and FOXP3+ were measured. The results indicated a high number of male patients in the age range of 21 to 30 years (33.3%) and a high percentage of female patients in the age group of 10 to 20 years (30.3%), with no significant levels $P > 0.05$. When compared to the control group, the concentration of CD4+, CD39+, and FOXP3+ in Alopecia patients was significantly higher than in healthy individuals ($P < 0.05$). According to this study's findings, patients with AA had higher levels of CD4+, CD39+, and FOXP3+ in their blood.

Keywords: *Alopecia areata*, Treg cells, CD39, Foxp3.

INTRODUCTION

T-cell infiltrates and the generation of cytokines around anagen stage hair follicles are two features of the organ-specific autoimmune illness *Alopecia areata* (AA). The anagen hair bulb's normal immune privilege collapsing may be a major factor in the etiology of this illness. Autoimmune illnesses have been linked to changes in the specialized CD4+ T cell population known as T helper cells and regulatory T cells (Tregs) (Loh et al., 2018).

In order to maintain immunological homeostasis and provide both self- and non-self-antigen immunological tolerance, a subpopulation of T cells called regulatory T cells (Tregs) controls immune responses (Selck & Dominguez-Villar, 2021). 4–10% of the CD4+ T-cell population in peripheral blood are naturally occurring Tregs (nTregs). They exert an anergic action on T effs and play a crucial role in peripheral immune tolerance (Garcia Caratozzolo, 2019). Psoriasis, *Alopecia areata*, Vitiligo, Pemphigus and Pemphigoid, and Systemic sclerosis are only a few autoimmune skin illnesses that can be brought on by Treg insufficiency (Mukhatayev et al., 2021).

The majority of the cellular infiltration surrounding the damaged HF in AA is made up of CD4+ and CD8+ T-cells. Surprisingly, CD4+ cells make up 60–80% of the lymphocytic infiltration in skin that has been exposed to AA (Ghraieb et al., 2018). Important elements in the etiology of AA include how Nave CD4+ can differentiate, regulatory T-cells that are CD4+ CD25+ (Tregs), which functionally suppress any detrimental activity, or become effector inflammatory regular T helper cells (Teff), like Th1, Th2, or Th17. Finding out which CD4+ cell subset contributes

most to AA pathogenesis and if it affects IP collapse is also crucial.

Effector T cell activity is inhibited by pericellular adenosine, which is produced by Tregs that express the ectoenzymes CD39 and CD73 and binds to the Adenosine Receptor (A2AR) (Steingold & Hatfield, 2020). Because the loss of these cells in AA patients' skin was consistent with the drop in circulating CD39+ Tregs, it is conceivable that the inflow of these cells into the skin makes the HF susceptible to an autoimmune reaction. Accordingly, the significant drop in CD39+ Tregs in the HF and the nearby unaffected skin may be a sign of non-lesional skin that is prone to Alopecia (Hamed et al., 2019).

The forkhead box P3 protein, often known as FOXP3, is involved in an immunological response. Both CD4+CD25+ and CD8+ T cells can express FoxP3 in humans (Singh & Bischoff, 2021). In the growth and operation of regulatory T cells, FOXP3, a member of the FOX protein family, functions as a signaling component and seems to act as a master regulator of the regulatory pathway (Dainichi & Kabashima, 2017). Systemic autoimmunity is brought on by loss of FOXP3 expression and/or deletion of the FOXP3 locus's TSDR, which is unique to Treg cells (Qu et al., 2022).

MATERIALS And METHODS

This investigation was conducted at a private dermatological clinic. 45 clinically diagnosed patients and 45 healthy controls from various regions of Iraq were included in the case study. Their ages ranged from 5 to 59. The samples were taken between the end of September 2021 and the beginning of January 2022. Patients gave their consent to take part in the trial. Patients with any autoimmune disease,

dermatological condition, or other disease who had received systemic or topical therapy prior to sample collection were also excluded from the study. Two groups made up the study groups. People with *Alopecia areata* included (45) patients (32 men, 13 women) ranging in age from (5-59) years. The control groups consisted of (45) individuals who appeared to be in good condition, ranging in age from 4 to 50 years, and who did not have any prior history of scalp or body lesions (25 males and 20 females). This study comprised all participants. Five milliliters of blood were drawn from the veins of each patient and the groups of healthy controls. A portion of the sample was placed in a gel tube and allowed to coagulate for around 30 minutes. Then, serum was isolated. The serum levels of CD4+, CD39+, and FOXP3+ were then quantitatively assessed using the Enzyme-Linked Immune Sorbent Assay (ELISA) technique. ELISA testing had been carried out in line with the manufacturer's instructions (Mybiosource, USA). Each well's optical density was assessed using an ELISA reader at 450 nm, and the results for the concentrations of CD4+, CD39+, and FOXP3+ were extrapolated from the standard curve. In order to conduct the statistical analysis for this study, IBM SPSS versions 25.0 were employed (Corporation, 2017)., the Data should be tested for normal distribution. homogeneity and randomization were calculated. Additionally, Pearson's chi-square test was used to establish the significant differences for non-parametric data while, mean and standard deviation, T-test, table of ANOVA and Pearson correlation were used to determine the distinctions that are substantial for parametric data.

RESULTS

This current work included 45 patients and 45 healthy control groups. From the 45 patients 32 (71.1%) were male, 13(28.9%) female of types of AA. Males were more frequently observed than females among *Alopecia areata* as in (Table 1). Results among studied groups showed an increased mean of CD4+(4.06±0.93) ng/ml and healthy control (3.18±0.65) ng/ml, CD39+(3.9±2.33) ng/ml and healthy control (1.59±0.61) ng/ml, and FOXP3+(5.12±2.24) ng/ml and healthy control (2.08±0.87) ng/ml respectively. The results showed significantly increased in serum of CD4+,CD39+,and FOXP3+ mean levels between patients and healthy control groups. With high significant relation (P< 0.05), for all parameters.

Table 1: Distribution of the gender of control and Alopecia patients

		case			P. value	
		Control	Patient	Total		
Gender	Female	N	20	13	0.095	
		%	44.4%	28.9%		36.7%
	Male	N	25	32		57
		%	55.6%	71.1%		63.3%
Total		N	45	45	90	

	%	100.0%	100.0%	100.0%	
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Table 2: Showed distribution of age groups and gender of Alopecia patients and control groups.

Age		Gender			P VALUE
		male	female	Total	
<10	N	13	10	23	0.6
	%	22.8%	30.3%	25.6%	
11-20	N	8	7	15	
	%	14.0%	21.2%	16.7%	
21-30	N	19	7	26	
	%	33.3%	21.2%	28.9%	
31-40	N	10	5	15	
	%	17.5%	15.2%	16.7%	
>41	N	7	4	11	
	%	12.3%	12.1%	12.2%	
Total		N	57	33	90
		%	100.0%	100.0%	100.0%

The table (2) showed the age group 21-30 years have a high percentage 33.3% of male and age group < 10 y have a high percentage 30.3% of female patients, with no significant levels P> 0.05.

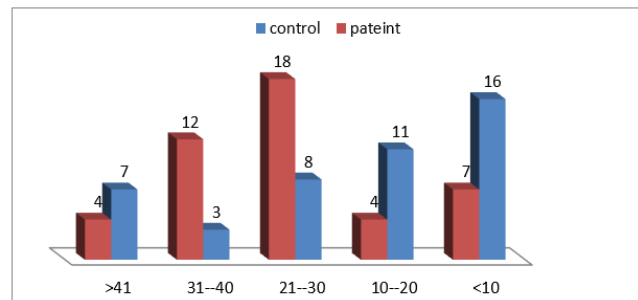


Figure 1: Showed distribution of the age group of control and Alopecia patients

Table 3: Descriptive statistics of the variables in the studied groups

Variable	N	Mean	Std. Deviation	Std. Error	P. value
CD4+ Control	45	3.18527	.650231	.096931	.01

	Patient	45	4.06162	.933210	.139115	
	Total	90	3.62344	.913088	.096248	
CD39+	Control	45	1.59604	.618596	.092215	.01
	Patient	45	3.90000	2.334064	.347942	
	Total	90	2.74802	2.055350	.216653	
Foxp3+	Control	45	2.08051	.878487	.130957	.00
	Patient	45	5.12182	2.241550	.334151	
	Total	90	3.60117	2.281218	.240461	

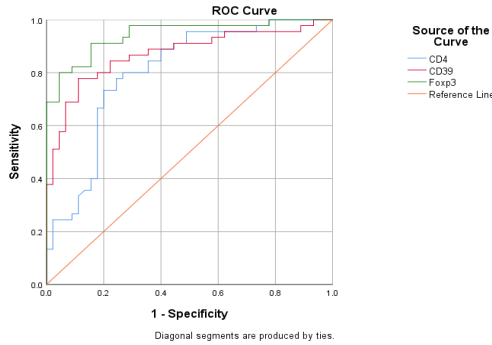


Figure 2: Showed distribution of the immunological markers of control and Alopecia patients

DISCUSSION

Autoimmune response brought on by the loss of the hair follicle immunological privilege, Immune privilege collapse is assumed to be either a primary event that triggers antigen presentation in a disturbed hair follicle environment or an event that occurs as a result of dysregulation of the central immune system that involves the follicles (Bertolini *et al.*, 2020). regulatory T cells are a subset of T lymphocytes (Tregs) controls immunological responses in order to preserve immunological tolerance to both self- and non-self-antigens and immune homeostasis. Treg populations and functions have been found in a rising number of autoimmune disorders. Numerous autoimmune skin conditions, such as Vitiligo, *Alopecia areata*, Pemphigoid and Pemphigus, Psoriasis, and Systemic sclerosis, can be brought on by Treg insufficiency (Mukhatayev *et al.*, 2021).

Alopecia sufferers have much more CD4+ T cells than healthy individuals, according to studies. In spite of the fact that the precise origin of *Alopecia areata* is unknown, mounting evidence indicates to changes in CD4, CD8 T cells and their cytokines as the key contributors to hair loss. Because AA skin lesions show per bulbar lymphocytic infiltration, the control group, which was made up of intra-follicular CD8+ T cells and perifollicular CD4+ T cells, formed a "swarm of bees" with elevated counts of telogen hairs in the acute and chronic stages, catagen hairs in the subacute stage, and miniature hairs in the chronic stage. This suggests that the activation of follicular autoantigen is necessary (Aldaffaie *et al.*, 2022). For the immune system's

homeostasis and maintenance of immunological tolerance, FOXP3+ regulatory T (Treg) cells are crucial. The molecular processes influencing the stability, adaptability, and functional activity of Treg cells have been the subject of extensive research in recent years. Autoimmune follicular antigen is caused by an imbalance in immunological tolerance in Alopecia (Wing *et al.*, 2019).

The suppressive activity of Tregs has been linked to CD39, which is largely found on the surfaces of endothelial cells and circulating platelets (Zeng *et al.*, 2020). Uncertainty persists over CD39's pathogenic significance to the Foxp3+ population's stability. As a result, it is challenging to identify other research that support or contradict this one. The fact that our study is unique in this field is crucial to note.

Compared to healthy and non-lesional tissue, the distribution of Treg subgroups in AA patients with lesions is noticeably abnormal. When compared to the healthy group, the FOXP3+ cells in the Alopecia patient group dramatically increased, and the CD39+ T regulatory cells also significantly increased ($P < 0.05$) in the Alopecia patient group. In contrast, AA patients with active hair loss had 90% fewer FOXP3 Treg cells in lesional skin and 75% fewer in non-lesional skin as compared to control healthy skin. (Åstrand *et al.*, 2018) The rise in CD39 expression on T-cells in people with active hair loss is an intriguing discovery in our understanding of the immunology of AA. This data raises the possibility that the disease might be managed by CD39+ Treg reconstitution (Raffin *et al.*, 2020).

(Younes *et al.*, 2022) According to this study, the level of CD4 T cells was assessed throughout the disease's active stage. There were no appreciable differences ($p > 0.05$) found for the immunological biomarker CD4 in blood between sick groups and healthy individuals in a prior study contradicting this one (Aldaffaie *et al.*, 2022). (Hamed *et al.*, 2019) disagree to increase CD39 in our study and also partially disagree with our study where they decrease in FOXP3 Treg in lesional skin of AA patients, but they concur to increase the serum level of Foxp3+ in patients in this study by reporting that Patients with *Alopecia areata* have higher levels of FOXP3+ CD39+ T regulatory cells. The current study differs from a prior work by (Hamed *et al.*, 2019) that claimed AA patients have higher CD39+ Treg cells, however our findings are consistent with that study in part because of a decrease in FOXP3+ value. (Elela *et al.*, 2016) further supported our results by demonstrating that patients with AA had modestly higher serum levels of Foxp3+.

CONCLUSION

In conclusion, the current work included 45 patients and 45 healthy control groups. From the 45 patients 32 (71.1%) were male, 13(28.9%) female of types of AA. with ages range from 5-59 year from different geographic areas in Iraq. The samples were collected during the period from the end of September 2021 to the January 2022. Consent was

taken from patients to participate in the study. Patients who had taken systemic or topical therapy before collecting the sample and those with any dermatology disease, autoimmune disease and other disease were excluded from study. Males were more frequently observed than females among *Alopecia areata* patients. circulating serum levels significantly increased of CD4+,CD39+,and FOXP3+ mean levels in patients compared to healthy control groups, with high significant relation, for all parameters.

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