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Original Article

Investigation of Level of Pleiotropic Cytokine (IL15) in Serum of *Alopecia Areata* Patients in Iraq

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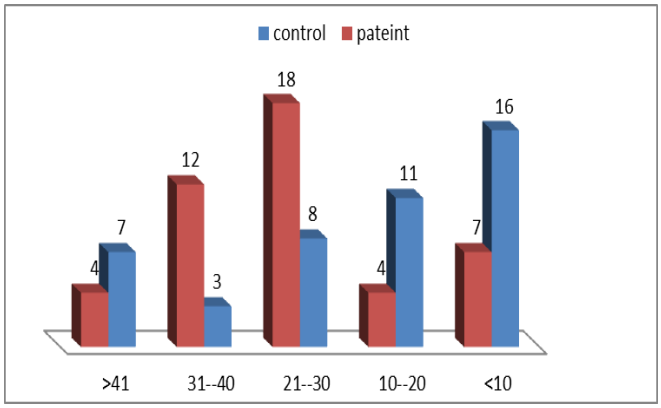
ABSTRACT

An autoimmune condition known as *Alopecia areata* (AA) affects the hair follicles and any part of the body with hair that results in patches of noncicatricial hair loss. This study was designed to investigate the role of IL-15 as inflammatory marker and determine the correlation of IL 15 with progression of *Alopecia areata.* This study was done at the dermatology private clinic. Case study included (45 clinically diagnosed patients and 45 healthy controls); with age ranges of 5-59 years old from different geographic areas in Iraq. IL-15 and was done by using ELISA. The results showed that the age group (21-30) years old have a high percentage 33.3% of male and age group < 10 years old have a high percentage 30.3% of female patients with no significant levels P> 0.05. The concentration of IL15 in Alopecia patients vs. the healthy persons have a slightly elevated according to control group, with high significant relation (P< 0.05). This study concludes that the circulating levels of IL-15 marker were elevated in patients with AA.

GRAPHICAL ABSTRACT



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Introduction

Any hair-bearing portion of the body can develop patches of noncicatricial hair loss due to the autoimmune disease *Alopecia areata* (AA). It is frequently associated with other autoimmune disorders, the most prevalent of which being atopy and autoimmune thyroiditis [1]. The disease impacts the hair-bearing regions of the head as well as other body parts by attacking the epithelium of hair follicles. According to [2], the AA prevalence in the general population varies from 0.1% to 0.2% in 2021, with a lifelong risk of 1.7%.

There are several elements that affect how well the hair bulb can resist immune system responses to self-keratinocyte and/or self-melanocyte peptides [1]. NK cells, CD8+ T cells, and specific subsets of CD4+ T cells all have the surface receptor NKG2D. These cells become active when their ligands activate this receptor [3].

To date, AA has affected 2% of the general population, affecting people of all ages, genders, and ethnicities [4]. The AA etiopathogenesis has undergone new research in the areas of genetics, immunology, oxidative stress, microbiome, allergies, microbiota, epigenetics, and other variables [5]. Cytokines are potent, highly specialized regulatory chemicals that primarily affect cells in a narrow range [6]. Multifunctional and essential for hematopoiesis, immunity, tissue repair, growth, and cellular development are cytokines [7].

Cytokines are regulatory proteins produced and secreted by lymphocytes and monocytes [8]. Interleukin-15 is a pleiotropic cytokine that affects many bodily cell types in various biological ways. It has an impact on the innate and adaptive immune system activities, and as a result, plays a crucial role in inflammation and immunological responses to infections and infestations. The AA in the mouse model of the disease developed was inhibited when the IL-15 receptor beta (IL-15R) was suppressed, suggesting that IL-15 plays a role in the AA pathogenesis [9].

IL-15 utilizes the transcription activator STAT-5 and the Janus (JAK) -1, Jak3 pathway to carry out its actions [10]. IFN- and IL-15 are promising therapeutic targets for *Alopecia areata* therapy [11]. However, the number of research done so far prevents conclusions on the use of IL-15 as a therapeutic point from being made [10].

The study's hypothesis is that AA has higher serum levels of IL-15, which reduces the inhibitory impact of regulatory T cells and prompts CD8+ NKG2D+ T cells to assault the cells in the hair bulb and start AA.

In this investigation, we measured the blood IL-15 levels in AA patients to see if rising levels would be a useful indicator of disease activity and severity.

Materials and Methods

Patient group

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 were created out of the research groups. Patients with *Alopecia areata* included (45) patients (32 men, 13 women) in the age range of (5-59) years old.

Healthy control group

The control groups contained (45) individuals who appeared to be in good health of both sexes (25 males and 20 females) and were between the ages of 4 and 50. They had no prior history of scalp or body lesions. The study included each and every participant.

Laboratory analysis

Each patient, along with groups of healthy controls, had five milliliters of venous blood drawn. Samples were placed in gel tubes and allowed to coagulate for around 30 minutes.

Serum was then divided. After that, the serum levels of IL-15 were quantitatively analyzed using the Enzyme-Linked Immune Sorbent Assay (ELISA) technique, and ELISA tests had been carried out in accordance with the manufacturing guidelines. After that, it was maintained at -20 °C until use in analysis (Mybiosource, USA). The ELISA reader evaluated the optical density of each well at 450 nm, and the results of the interpolation of the IL15 concentration from the standard curve were determined.

Statistical analysis

To conduct the statistical analysis for this study, IBM SPSS versions 25.0 were employed (Corportation, 2017). The Data should be tested for normal distribution. The homogeneity and randomization were calculated. In addition, 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 as in the Table 1.

Results and Discussion

This current work included 45 patients and 45 healthy control groups. From the 45 patients 32 (71.1%) were males, 13 (28.9%) females of the AA types. Males were more frequently observed than females as in Table 2 with no significant levels $P > 0.05$.

Table 1: Descriptive statistics of the (T-Test) of IL15 in the studied groups

Variable	Patients group (n = 45) Mean±SD	Control group (n = 45) Mean±SD	P by t test
IL15	18.4±4.4	17.5±3.8	0.01

Table 2: Distribution of the gender of control and Alopecia patients

			case		Total	P-value
			Control	Patient		
Gender	Female	N	20	13	33	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%	

Table 3: Distribution of age groups and gender of Alopecia patients and control groups

			Gender		Total	P-value
			Male	Female		
Age	<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%	

Table 3 showed the age group 21-30 years old has a high percentage 33.3% of males and age group < 10 years old has a high percentage 30.3% of female patients with no significant levels *P*> 0.05. This table showed the severity classify of Alopecia patients and age groups, (21-30 years old) have a high number within universal severity (3 patients), and mild severity (15 patients) with no significant levels > 0.05.

In this study, it showed the IL15 concentration of Alopecia patients vs. the healthy persons have a slightly elevated according to control group as in Figure 2 with high significant relation (P< 0.05). Table 4 showed the severity classify of Alopecia patients and age groups.

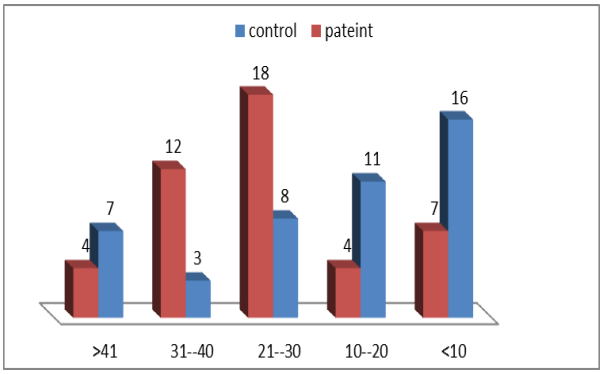

Figure 1: Showed distribution of the age group of control and Alopecia patients

Table 4: Distribution of the disease severity according to age groups of Alopecia patients

		Age groups					Total
		<10	11-20	21-30	31-40	>41	
Severity Disease	Universal	1	1	3	1	1	7
	Moderate	1	0	0	1	0	2
	Mild	5	3	15	10	3	36
Total		7	4	18	12	4	45
P-value		> 0.05					

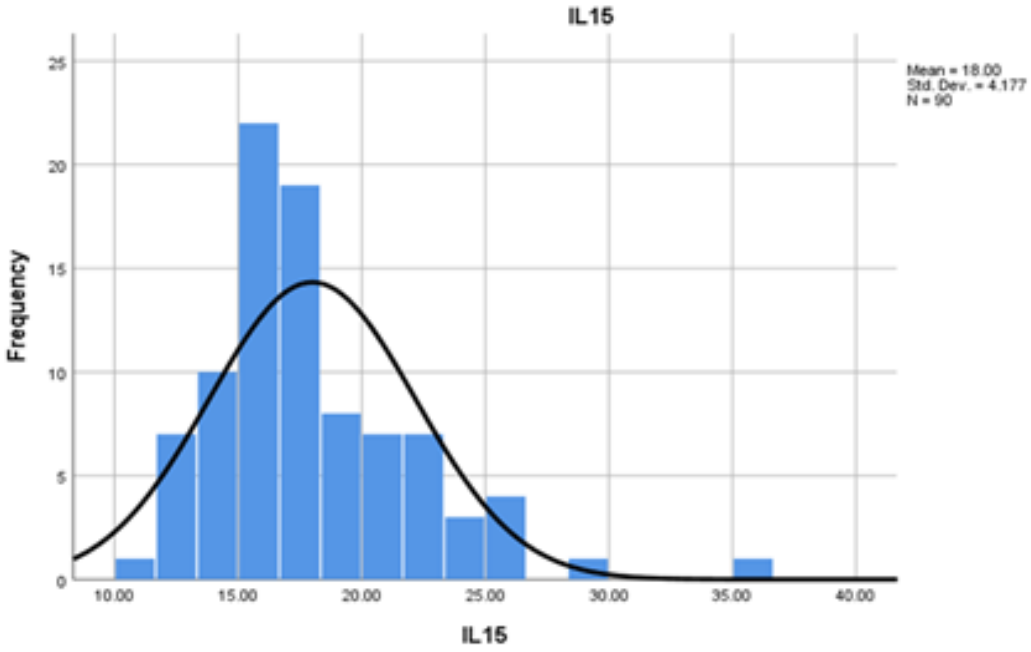

Figure 2: Normal distribution of IL15 applied in this study

Interleukin-15 (IL-15), a pleiotropic cytokine, affects numerous cell types biologically in different ways. It has a substantial impact on the immune system's response to infections, infestations, and inflammatory processes by influencing both the innate and acquired immune system activities. In addition, it suppresses pro-apoptotic proteins while increasing anti-apoptotic ones, which promotes the survival of T, B, and NK cells and serves as a growth factor. Numerous cells widely express it [12]. Numerous inflammatory and autoimmune conditions, including rheumatoid arthritis, SLE, polymyositis, and dermatomyositis have been linked to it [13]. Its involvement in a few dermatologic conditions has been researched. Memory CD8+ T cell expansion, maintenance, and proliferation depend on IL-15. The main source of INF-, which is primarily responsible for IP collapse and the AA formation, is memory CD8+ T cells [14].

Table 5: Correlation of IL15 and disease severity

IL15 and disease severity correlation							
IL15 concentration			Disease severity			Total	P-value
			Universal	Mild	Moderate		
	<14	N	0	3	0	3	0.25
		%	0.0%	6.7%	0.0%	6.7%	
	14-18	N	3	21	1	25	
		%	6.7%	46.7%	2.2%	55.6%	
	19-22	N	4	8	0	12	
		%	8.9%	17.8%	0.0%	26.7%	
	>23	N	0	4	1	5	
		%	0.0%	8.9%	2.2%	11.1%	
Total		N	7	36	2	45	
		%	15.6%	80.0%	4.4%	100.0%	

In this study, it showed a low significant relation of IL15 with disease severity (P> 0.05).

IL-15 not really related with disease severity since its lower in group with moderate than the group with mild disease as in Table 5. The results of the current study showed that serum IL-15 levels were significantly higher in patients with AA compared with serum levels in the healthy control group because the IL-15concentration rose directly in correlation with the area of Alopecia, reaching its highest value in patients with total alopecia. This was found to be true, in contrast to [15], who did not find a significant difference between the serum levels of patients and the healthy control group.

The IL15 serum levels are significantly greater in the ill group than in the control group (P= 0.001), according to the findings of the earlier studies [16] and these findings were consistent with those of the current investigation. A recent study [12] that found that serum levels of IL-15 were significantly higher in patients with AA and revealed a strong positive link with the severity and activity of the disease supports the findings of this investigation. The effectiveness of serum IL-15 in identifying AA patients from controls was evaluated using the receiver operating characteristic curve. Clinical experiments targeting the IL-15 pathway have used mutant IL-15, compounds that disrupt the soluble IL-15 receptor chain, antibodies directed against the IL-15 cytokine, and the IL 2R/IL-15R component required by IL 2 and IL-15. This is owing to the fact that elevated IL-15 expression has been connected to various inflammatory autoimmune diseases. Inhibiting the IL-15 receptor prevents delayed-type hypersensitivity [17].

Clinical investigations suggest that targeting the IL-15 pathway may help treat a number of diseases, including vitiligo, rheumatoid arthritis, and plaque psoriasis [18]. Concerning AA, JAK inhibitors were found to ameliorate AA symptoms [9]. They suggested a number of mechanisms by which these drugs operate, including blocking pSTAT5 activation brought on by IL-15 and IL-15-induced elevation of Granzyme B and IFN- production. All of these findings suggest that IL-15-targeted biologic

therapy is a novel approach for the treatment of a wide range of inflammatory illnesses. The IL-15level in the serum could be a reliable sign of AA activity. Targeting this molecule could result in a recovery from this condition.

Conclusion

The combination therapy of vitamin C and thiamine does not improve treatment outcomes. Thiamine and ascorbate alone showed better benefits compared with combination therapy viewed from the incidence rate, MMP-9/TIMP-1 ratio, and SOFA score.

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Authors' contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

Conflict of Interest

The author declared that they have no conflict of interest.

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