Egyptian Journal of Medical Research

Print ISSN: 2682-4396 / Online ISSN: 2682-440X

Original article

DETECTION OF TISSUE INTERLEUKIN-15 AND INTERFERON GAMMA IN ALOPECIA AREATA

Yasser Mostafa Gohary [a] Mona Ahmed Mandour Ahmed Negm [a], and Sahar Aly Daoud [b], Hanan Abdelrazik Kamel [a]

[a] *Dermatology & Venereology department, Faculty of Medicine, Beni-Suef University, Egypt*

[b] *Pathology department, Faculty of Medicine, Beni-Suef University, Egypt*

Article Info

Article history:

Received 10 July 2023

Accepted 16 July 2024

Corresponding Author:

Mona Ahmed Mandour Ahmed

monanagm55@gmail.com

Keywords

IL15,

IFN-γ,

Alopecia Areata (AA).

Abstract:

The current study was designed with an aim to evaluate immune staining expression of IFN-γ and IL-15 in patients with AA (between lesional and non-lesional skin biopsies) assessing their relationship to clinical parameters of the diseases. It was a case-control study, including thirty-six patients with localized, recent onset AA, a 3.5 mm punch skin biopsies were taken from lesional and non-lesional skin 5 cm away from the lesion of every patient as a control group for immunohistochemical analysis of IL15 and IFN-γ. The level of IL15 and IFN-γ show correlation with the severity of inflammation as they were higher in the more severe cases and who has multiple lesions. They show non-statistically differences between healthy skin biopsy and lesional skin biopsy. There were neither affected by the subject's age and site and extent of lesion. We can conclude that skin of severe cases of AA has a high level of IL15 and IFN-γ than localized and mild cases.

1. Introduction:

Hair loss on the scalp or elsewhere on the body is the hallmark symptom of alopecia areata (AA), a diverse condition that can affect anyone. The hair follicles are the target of the autoimmune disease alopecia areata, which causes non-cicatricial bald patches to appear anywhere on the body where hair normally grows. Asthma and autoimmune thyroiditis are the most frequently seen related autoimmune disorders [1]. A wide range of clinical presentation can occur-from a single patch of hair loss (alopecia unilocularis, AUl), multiple patches (alopecia multilocularis, AM) to complete loss of hair on the scalp (alopecia totalis, AT) or the entire body (alopecia universalis, AU) [2]. Alopecia areata affects approximately 2% of the general population at some point during their lifetime [3]. Alopecia areata can appear at any age, however it typically appears between the ages of 25 and 36, with a mean age of onset somewhere in the middle [4]. Alopecia areata that begins in childhood (between the age range of 5-10 years) typically manifests as a more severe form, such as alopecia universalis [5]. The pathogenesis of AA is not completely clear yet, however, it is well established that genetic and environmental factors contribute to its development [6]. Now, the autoimmune attack of the hair follicles due to the collapse of its ability to tolerate the introduction of antigens without eliciting an inflammatory immune response (The immune privilege) is considered to play the main role in AA development [7]. The histopahtologic features of the AA consist of perifollicular lymphocytic infiltrates around anagen hair follicles, consisting of both CD4+ and intrafollicular infiltrates of CD8+ cells [8]. Although the etiopathogenesis of the disease is not clear, several studies have shown that within the cascade of pathogenesis of AA, cytokines play a crucial role. The immune response presented in AA is associated with aberrant lesional expression of interferon-gamma (IFN-γ), interleukin-2 (IL-2) and IL-1b, and overexpression of intercellular adhesion molecule-1 (ICAM-1) and major histocompatibility complex (MHC) molecules on hair follicle keratinocytes and dermal papilla cells [9]. IFN-γ is produced by perifollicular or follicular antigen presenting cells and among several actions it also deprives dermal papilla cells of their ability to maintain anagen hair growth [10]. The changes in serum IFN-γ concentrations were found in many diseases, such as psoriasis and systemic lupus erythematous. In some of these diseases, serum IFN-γ concentration correlated with activity and intensity of the disease, and may be used as a prognostic factor [11]. Interleukin-15 (IL-15) is a pleiotropic cytokine which exerts the multiple biological effects on different body cell types. It affects the functions of the cells of the immune system, both innate and adaptive,

and hence, it has an important role during inflammation and during the immune responses to infections and infestations [12]. The role of IL-15 in the pathogenesis of AA was suggested when the blocking of IL-15 receptor beta (IL-15Rβ) reduced the number of CD8+NKG2D+ T cells in the skin and prevented the development of AA in the mouse model of the disease [13].

The aim of our study is to evaluate immune staining expression of IFN-γ and IL-15 in patients with AA and healthy subjects assessing their relationship to clinical parameters of the diseases.

2. Patients and Methods:

2.1. Ethical Consideration:

The ethical committee of FM-BSU REC gave its approval to conduct the current study on 1st of September/2020, approval number: (FMBSUREC/01092020/Negm). A written informed consent was taken from every patient before participation in this study after the study's goals were clarified. The confidentiality of the data base was ensured.

2.2. Patients:

This study had been conducted at Beni-Suef university hospital. It was a case-control study included 36 patients with AA, a 3.5 mm punch skin biopsies were taken from lesional and non-lesional skin 5 cm away from the lesion. Patients with Alopecia Areata were included in the study while those with other hair disorders, suffering from concomitant infectious, inflammatory or autoimmune cutaneous or systemic disease and those who had received any treatment within previous 3 months.

2.3. Methods

Every patient was subjected to history taking including personal history (age, sex, sociodemographic data), present history including duration of the disease, previous and current treatment, associated diseases (thyroid disease, diabetes mellitus or others), and family history of AA. Scalp was examined to assess sites, number, and size of alopecia patches. The body was carefully examined to detect any alopecia patches in any hairy area. Nail examination was carried out to detect any nail involvement. Tissue sample was collected from every participant in the study under complete aseptic precaution. Punch biopsy was taken, and the blade size was 3 (mm). Each biopsy was immediately fixed 10% neutral buffered formalin for 24 hours, and routinely processed to paraffin embedding. Sections prepared from paraffin blocks were stained by Hematoxylin & Eosin method for routine Histopathological examination. Immunohistochemistry was performed using Dako autostainerlink 48. Four µm thick sections from each case were incubated with rabbit polyclonal anti- IL15 antibody (Catalogue No. YPA2162, Chongqing Biospes Co., Ltd, China) and rabbit polyclonal anti- IFN- γ antibody (Catalogue No. YPA2285, Chongqing Biospes Co., Ltd, China). Positive and negative controls consisted of lung and placental tissues stained

with and without primary antibody, respectively. All slides were examined by light microscopy (Olympus CX23) and all included photos were taken by Leica digital pathology slide scanner (APERIO LV1) at pathology lab, Beni-Suef University hospital. Results of the two groups were compared.

2.4. Data management and Statistical Analysis:

The collected data was coded, entered, and analyzed using the IBM software statistical package for social sciences (SPSS) (version 25). For categorical variables, descriptive statistics were in the form of frequency and percentage, while for numerical variables in the form of mean and standard deviation. (mean ± SD). The proper statistical significance measures were used: (Kruskal-Wallis, Wilcoxon's, Chi square, logistic regression analysis, and Spearman's correlation). Data were presented and suitable analysis was done according to the type of data (parametric and non-parametric) obtained for each variable. (Independent Sample t-test, Chi-Square ($\chi 2$) test and Pearson's correlation analysis; {r-values: 0 to 0.3 positive or negative (slight), 0.3 to 0.7 (moderate) and 0.7 to 1 (strong). Statistical significance was described at a p-value of less than or equal to 0.05. Simple charts were used to demonstrate some findings.

3. Results:

The age distribution of the studied participants was ranged from (19) to (70) with an average age of (31.52 ±9.7) years old. All the studied participants in the current study were males with negative family history of AA.

Table (1) demonstrates the description of AA lesion among studied participants. Twenty-two (61.1%) of the studied patients have AA lesion for the 1st time while fourteen (38.9%) have recurrent lesions. Regarding lesion number, half patients have single lesion and the other half have multiple lesions. SALT score was ranged from (0.18) to (0.90) with an average score of (0.38 ±0.24). Regarding disease duration it was ranged from (1) to (12) with an average disease duration of (4.16 ±3.84) months.

Figure (1) demonstrates comparison of immune staining expression of IFN-γ in skin lesions with AA and healthy skin. Score +4 (76-100%) was higher in skin lesions with AA as compared with normal skin (7 samples vs. 3 samples) but without a statistically significant difference. Score +3: 51-75% was similarly distributed in skin lesions with AA and normal skin (5 samples in each group). Score +2 (26-50%) was higher in skin lesions with AA as compared with normal skin (10 samples vs. 9 samples) but without a statistically significant difference. Score +1 (2-25%) was lower in skin lesions with AA as compared with normal skin (10 samples vs. 15 samples) but without a statistically significant difference. Negative score: (0-1%) was similarly distributed in skin lesions with AA and normal skin (4 samples in each group).

Table (1): Description of AA lesion among studied participants; (N= 36):

		Descriptive Statistics
1st time or recurrent; N (%)	**1st time**	22 (61.1)
	Recurrent	14 (38.9)
Lesion Number; N (%)	**Single**	18 (50.0)
	Multiple	18 (50.0)
SALT; mean ±SD		0.38 ±**0.24**
Duration (Months); mean ±SD		4.16 ±**3.84**

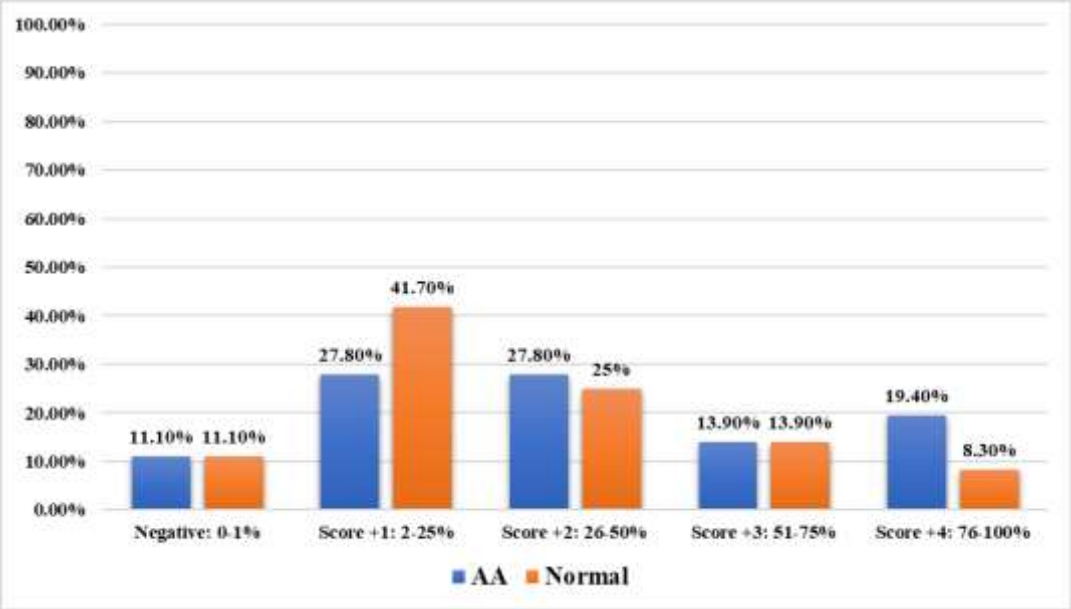

Figure (1): comparison of immune staining expression of IFN-γ in skin lesions with AA and healthy skin among studied participants.

As regard IFN-γ expression location in skin lesions with AA and healthy skin, the expression was more predominant in Upper dermis in both groups without a statistically significant difference (p-value >0.05). Comparison of immune staining expression of IL-15 in skin lesions with AA and healthy skin. Positive staining was slightly higher in AA skin lesions as compared with healthy skin (16 vs. 15 samples) but without a statistically significant difference (p-value>0.05).

Figure (2) demonstrate the association between immune staining expression of IFN-γ and IL-15 in skin lesions with AA. All the examined samples with IFN-γ Score +4 (76-100%) showed positive IL-15 expression with a statistically significant p-value =0.008 which mean that there is a statistically significant association between immune staining expression of IFN-γ and IL-15 in skin lesions with AA.

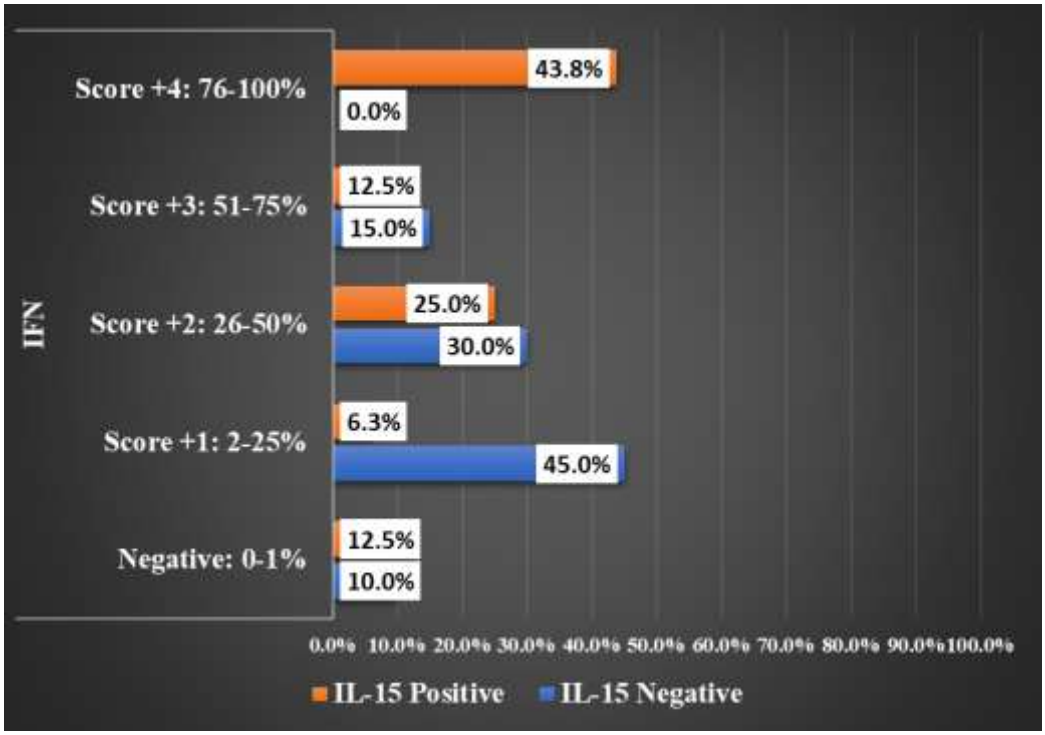

Figure (2): the association between immune staining expression of IFN-γ and IL-15 in skin lesions with AA.

As regards the association between SALT score with interleukin-15 tissue expression in AA patients, figure (3) demonstrates that SALT was significantly higher among studied samples with positive IL-15 expression. The mean ±SD SALT score was (0.48 ±0.28 vs. 0.29 ±0.14, p=0.014) in positive and negative IL-25 expression, respectively.

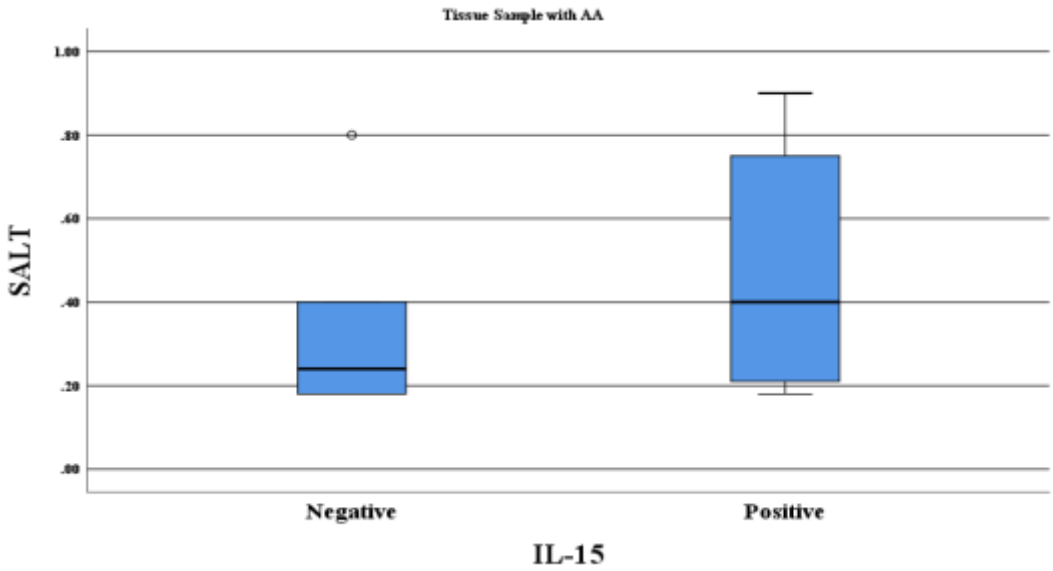

Figure (3): Association between SALT score with interleukin-15 tissue expression in AA patients

According to Spearman's rank correlation co-efficient analysis, as illustrated in table (2) there was a statistically significant positive linear correlation between SALT score and IFN-γ tissue expression in AA patients, (r= 0.615, p=0.001). there was non-statistically significant negative linear correlation between patients' age and IFN-γ tissue expression in AA patients, (p> 0.05). there was non-statistically significant negative linear correlation between disease duration and IFN-γ tissue expression in AA patients, (p> 0.05).

Table (2): Correlation between SALT score with IFN-γ tissue expression in AA patients; (N= 36):

		IFN-γ
SALT	**r**	0.615
	p-value	**0.001***
Age (years)	**r**	-0.102
	p-value	0.555
Duration (Months)	**r**	-0.226
	p-value	0.185

Figure (4) demonstrate comparison of Inflammation in H&E Section in skin lesions with AA and healthy skin. Severity of inflammation was increased significantly in AA skin tissue as compared with healthy skin (p-value =0.021).

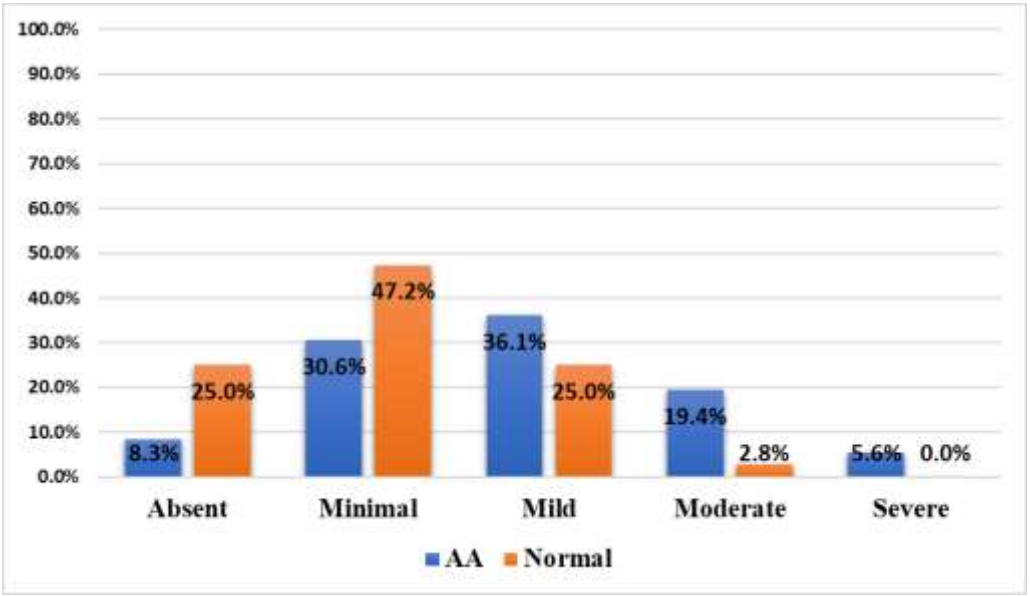

Figure (): Comparison of Inflammation in H&E Section in skin lesions with AA and healthy skin among studied participants.

3. Discussion:

Our study aimed to evaluate immune staining expression of IFN-γ and IL-15 in patients with AA and healthy subjects assessing their relationship to clinical parameters of the diseases. Immunostaining evaluation of for both IL15 and IFN-γ were studied on sections prepared from paraffin blocks. The current study was case-control study conducted on thirty patients with Alopecia Areata (AA), (30 Tissue samples from AA lesions and other 30 Tissue samples from healthy skin as control samples from the same patients). As regarding SOCIO DEMOGRAPHIC DATA, the age distribution of the studied participants that ranged from (19) to (70) with mean age of (31.52 ±9.7) years old. All the studied participants in the current study were males with negative family history of AA. Also regarding demonstrate the description of AA lesion among studied participants. Twenty-two (61.1%) of the studied patients have AA lesion for the 1st time while fourteen (38.9%) have recurrent lesions. Regarding lesion number, half patients have single lesion and the other half have multiple lesions.

AA is considered as a disease of all age group; however, most patients present at age of 21–40 years. Globally, AA onset was at 25 and 36 years of age in Singapore and USA, respectively [5]. AA has three main variants which are patchy AA (localized hairless areas), alopecia totalis (entire scalp affected), and alopecia universalis affecting all body surface area. Other AA subtypes include ophiasis (band-like alopecia in the occipital and temporal scalp), sisaipho (central hair loss sparing the marginal hair line), and diffuse form [13].

Our results were similar to [14], where A total of 216 patients with AA were included. The overall prevalence of AA was approximately 2.3%. The mean disease duration at the time of presentation was 2 months while the mean age of onset was 25.61 years. The most common type of AA in both adult and pediatric groups was the patchy type involving the scalp [15]. Comorbid diseases were found in 32.41% of patients. Common associated conditions included hypothyroidism, diabetes mellitus, and atopic diseases. Conclusion. The overall prevalence of AA among a population of Saudi patients is 2.3%. AA prevalence is higher in pediatrics than adults. Common comorbid conditions include hypothyroidism, diabetes mellitus, and atopic diseases.

As regards the SALT score, it is demonstrated in. SALT score was ranged from (0.18) to (0.90) with an average score of (0.38 ±0.24). Regarding disease duration, it was ranged from (1) to (12) with an average disease duration of (4.16 ±3.84) months. Also, as regard IFN-γ expression location in skin lesions with AA and healthy skin, the expression was more predominant in Upper dermis in both groups without a statistically significant difference (p-value >0.05). INF-γ is the main cytokine known

to be aberrantly expressed in AA through a CD4+ Th1-mediated response. INF-γ is predominantly produced by NK and natural killer T (NKT) cells as part of the innate immune response, by CD4 Th1 and CD8 cytotoxic T lymphocytes (CTL) and by effector T cells, once antigen-specific immunity develops [16].

Also, as regarding Comparison of immune staining expression of IFN-γ in skin lesions with AA and healthy skin among studied participants; (N= 36), there was no statistically significant difference between AA and healthy skin. Score +4 (76-100%) was higher in skin lesions with AA as compared with normal skin (7 samples vs. 3 samples) but without a statistically significant difference. Score +3: 51-75% was similarly distributed in skin lesions with AA and normal skin (5 samples in each group). Score +2 (26-50%) was higher in skin lesions with AA as compared with normal skin (10 samples vs. 9 samples) but without a statistically significant difference. Score +1 (2-25%) was lower in skin lesions with AA as compared with normal skin (10 samples vs. 15 samples) but without a statistically significant difference. Negative score: (0-1%) was similarly distributed in skin lesions with AA and normal skin (4 samples in each group). Anagen hairs have an immune privilege environment characterized by inhibition of major histocompatibility complex (MHC) class expression along with high expression of guardians like transforming growth factor (TGFβ1/2). According to the current accepted theory, the occurrence of AA needs a collapse of immune privilege through increased production of INF-γ7,8 followed by T-cell activation and hair follicle destruction [17].

In a study done by [18], they evaluated Interferon- gamma serum level and immunohistochemical expression of CD8 cells in tissue biopsies in patients with alopecia areata in correlation with trichoscopic findings. The study included 30 patients with AA and 15 age- and sex-matched healthy controls. Trichoscopy was performed and photographs were captured for the alopecic areas, and the enzymelinked immunosorbent assay technique was used for serum level of IFN-γ assessment and immunohistochemistry for CD8 cells. The results obtained indicate that IFN-γ serum level in patients was significantly higher than that of control subjects, and significantly correlated with the activity status and the duration of the disease. CD8+ T cells infiltrate intensity significantly correlated with severity. Yellow dots (YDs), vellus hair, black dot, and exclamation marks were the most common trichoscopic findings. The presence of black dots significantly correlated to the disease activity, duration, serum IFN-γ, and CD8+ infiltrate intensity. The presence of YDs significantly correlated with the mean serum IFN-γ level. Exclamation marks significantly correlated with the disease activity and the

degree of CD8+ infiltrate. In conclusion, trichoscopy could be a reliable indicator of the IFN-γ serum level and CD8+ T cell infiltrate intensity in AA patient. This study differs from our study in that they evaluated serum IFN-γ which may be more important than tissue IFN-γ in pathogenesis of AA.

Hence, IFN-γ production, although an important factor in disease initiation, it may have to be associated with other cofactors that determine disease extent. In the same way, IFN-γ production is not significantly correlated with the tendency of the disease to recur as a single event. The latter may be attributed to the nature of the study being a cross-sectional investigation, where the fate of first attack cases is not known. These results match with those of [19] who reported significant difference in serum levels of IFN-γ between patients with patchy AA and control group [20]. On the other hand, the study of [21] showed no significant difference in serum levels of IFN-γ between patients with patchy type and the control group.

In our study, as regarding comparison of immune staining expression of IL-15 in skin lesions with AA and healthy skin. Positive staining was slightly higher in AA skin lesions as compared with healthy skin (16 vs. 15 samples) but without a statistically significant difference. Also, as regarding the association between immune staining expression of IFN-γ and IL-15 in skin lesions with AA. All the examined samples with IFN-γ Score +4 (76-100%) showed positive IL-15 expression with a statistically significant p-value =0.008 which mean that there is a statistically significant association between immune staining expression of IFN-γ and IL-15 in skin lesions with AA.IL-15 acts as a pro-inflammatory cytokine and stimulates autoreactive T cells. IL-15 acts through the Janus (JAK) -1, Jak3 pathway and uses the transcription activator STAT-5. Both IFN-γ and IL-15 are promising therapeutic targets in the treatment of alopecia areata [22].

In the study of [23], they concluded that the concentration of IL-15 in patients with alopecia areata was significantly higher than in the control group. Moreover, the concentration of IL-15 increased in direct proportion to the area of alopecia, the highest value in patients with total alopecia. For this reason, it is important to search for new medical treatments that will enable patients to stay physically healthy, and what is equally important, to remain mental health. JAK inhibitors mediate IL-15 signal transduction, they may be taken in consideration as a possible therapeutic option. This may prevent the development of alopecia areata. JAK inhibitors represent a promising therapeutic option for people with alopecia areata. However, none of them (Tofacinib, Ruxolinib, Baricitinib) have been approved by the FDA for this indication, so far [24]. The effect of ruxolitinib on human dermal papilla cells was observed in a study, for this purpose, an in vitro model of previously interferon

gamma-exposed cells was used, and the expression of the wnt/beta catenin pathway, JAK-STAT pathway and growth factors was examined using rt-PCR and Western Blot [25]. Ruxolitinib did not affect cell viability, and activated some components of the wnt-beta catenin pathway, inhibited DKK1 expression, and also reversed IFN-dependent expression of a number of molecules, such as caspase-1, IL-1beta, IL-15 and IL-18, as a result reducing their expression. The drug increased the expression of some growth factors, and also inhibited the phosphorylation of JAK1, JAK2, JAK3, STAT1 and STAT3. Study results suggest that ruxolitinib therapy may prevent interferon gamma-induced hair loss. As regards the association between SALT score with interleukin-15 tissue expression in AA patients, SALT was significantly higher among studied samples with positive IL-15 expression. The mean ±SD SALT score was (0.48 ±0.28 vs. 0.29 ±0.14, p=0.014) in positive and negative IL-25 expression, respectively. Also was a statistically significant positive linear correlation between SALT score and IFN-γ tissue expression in AA patients, (r= 0.615, p=0.001). These results are consistent with [26], they included 42 patients (children) with alopecia areata, and 37 healthy children. Peripheral venous blood samples were collected from all participants to measure the concentration of the following cytokines - IL-6, IL-15, IL-17A and IFNgamma using the ELISA method. There was a significant increase in the concentration of all of the tested cytokines in the test group compared to the control group. Moreover, the concentration of IL-15 increased in direct proportion to the length of the disease, while the concentration of IL-17A decreased with the over two years long episode of disease and in the presence of thyroiditis. The highest mean concentration in the group of patients with alopecia areata among the tested cytokines was IFN-gamma (158.70 pg/ml), while IL-15 was in second place (89.59 pg / ml).

In a study done by [27], they stated that. The most evidence-based hypothesis is an autoimmune reaction caused by the collapse of hair follicle immune 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. Several gene loci have been identified with alopecia areata. The key immune effectors in the pathogenesis include autoreactive effector T cells, natural-killer group 2, member (NKG2D) + CD8+cytotoxic T cells, natural killer (NK) cells, Janus kinase, signal transducers, and activators of transcription (JAK/STAT) pathway, MHC-I chain-related gene A (MICA), interferon- γ (IFN- γ) and interleukin-15 (IL-15). Finally, they concluded that, alopecia areata has no accepted cure and has an unpredictable response to treatment. The recognition of the exact pathogenic

mechanisms of alopecia areata is necessary to identify the potential therapeutic targets.

The current study showed some limitations, a small sample size and the use of normal skin as a control for the same patient, in order to know IL-15 and IFN-γ exact role in AA, it was better the normal skin samples to be taken from matched healthy controls rather than the same patients.

The elevated tissue level of IL-15 and IFN-γ in patients with active AA might suggest its role as an important signaling cytokines in the pathogenesis of AA. There levels are influenced by disease severity which might reflect disease prognosis. Further studies on a larger sample size are needed to clarify the role of IL-15 and IFN-γ in AA. Recent studies on JAK inhibitors, either a systemic or topical form, targeting pathogenic molecules including IL-15 are now gaining interest in order to prove its safety and efficacy in treatment of AA.

Acknowledgements:

" to all the ladies who contributed with us to our current study"

Authors' contributions:

MM, HA: sampling, performing the experiments, data analysis, preparing the first draft of the manuscript. YM: supervision, statistical analysis, and revision of the manuscript. SD: conceptualization, supervision, data analysis, and preparing and revising the manuscript. All authors have read and approved the final manuscript.

Funding:

None.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate.

The Research Ethical Committee of Faculty of Medicine, Beni-Sueif University, has given the approval on conducting the present research.

Consent for publication:

The written informed consent was obtained from all patients prior to the study.

Competing interests:

The authors declare that they have no competing interests.

4. References:

1. Seetharam KA. Alopecia areata: an update. Indian J Dermatol Venereol Leprol. 2013 Sep-Oct;79(5):563-75. doi: 10.4103/0378-6323.116725. PMID: 23974575.
2. Pratt CH, King LE Jr, Messenger AG, Christiano AM, Sundberg JP. Alopecia areata. Nat Rev Dis Primers. 2017 Mar 16;3:17011. doi: 10.1038/nrdp.2017.11. PMID: 28300084; PMCID: PMC5573125.
3. Agre K, McCarthy Veach P, Bemmels H, Wiens K, LeRoy BS, Hordinsky M. Familial implications of autoimmune disease: Recurrence risks of alopecia areata and associated conditions in first-degree relatives. J Genet Couns. 2020

Feb;29(1):35-43. doi: 10.1002/jgc4.1178. Epub 2019 Oct 11. PMID: 31605426.

4. Lepe K, Zito PM. Alopecia Areata. 2022 Aug 25. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan–. PMID: 30725685.
5. Juárez-Rendón KJ, Rivera Sánchez G, Reyes-López MÁ, García-Ortiz JE, Bocanegra-García V, Guardiola-Avila I, Altamirano-García ML. Alopecia Areata. Current situation and perspectives. Arch Argent Pediatr. 2017 Dec 1;115(6):e404-e411. English, Spanish. doi: 10.5546/aap.2017.eng.e404. PMID: 29087123.
6. Darwin E, Hirt PA, Fertig R, Doliner B, Delcanto G, Jimenez JJ. Alopecia Areata: Review of Epidemiology, Clinical Features, Pathogenesis, and New Treatment Options. Int J Trichology. 2018 Mar-Apr;10(2):51-60. doi: 10.4103/ijt.ijt_99_17. PMID: 29769777; PMCID: PMC5939003.
7. Paus R, Bulfone-Paus S, Bertolini M. Hair Follicle Immune Privilege Revisited: The Key to Alopecia Areata Management. J Investig Dermatol Symp Proc. 2018 Jan;19(1):S12-S17. doi: 10.1016/j.jisp.2017.10.014. PMID: 29273098.
8. Kasumagic-Halilovic E, Prohic A, Karamehic J. Serum concentrations of interferon-gamma (IFN-g) in patients with alopecia areata: correlation with clinical type and duration of the disease. Med Arh. 2010;64(4):212-4. PMID: 21246917.
9. Ünlü B, Türsen Ü, Rajabi Z, Jabalameli N, Rajabi F. The Immunogenetics of Systemic Sclerosis. Adv Exp Med Biol. 2022;1367:259-298. doi: 10.1007/978-3-030-92616-8_10. PMID: 35286699.
10. Ryu S, Lee Y, Hyun MY, Choi SY, Jeong KH, Park YM, Kang H, Park KY, Armstrong CA, Johnson A, Song PI, Kim BJ. Mycophenolate antagonizes IFN-γ-induced catagen-like changes via β-catenin activation in human dermal papilla cells and hair follicles. Int J Mol Sci. 2014 Sep 22;15(9):16800-15. doi: 10.3390/ijms150916800. PMID: 25247578; PMCID: PMC4200814.
11. Gregoriou S, Papafragkaki D, Kontochristopoulos G, Rallis E, Kalogeromitros D, Rigopoulos D. Cytokines and other mediators in alopecia areata. Mediators Inflamm. 2010;2010:928030. doi: 10.1155/2010/928030. Epub 2010 Mar 11. PMID: 20300578; PMCID: PMC2837895.
12. Ebrahim AA, Salem RM, El Fallah AA, Younis ET. Serum Interleukin-15 is a Marker of Alopecia Areata Severity. Int J Trichology. 2019 Jan-Feb;11(1):26-30. doi: 10.4103/ijt.ijt_80_18. PMID: 30820130; PMCID: PMC6385510.
13. Xing L, Dai Z, Jabbari A, Cerise JE, Higgins CA, Gong W, de Jong A, Harel S, DeStefano GM, Rothman L, Singh P,

Petukhova L, Mackay-Wiggan J, Christiano AM, Clynes R. Alopecia areata is driven by cytotoxic T lymphocytes and is reversed by JAK inhibition. Nat Med. 2014 Sep;20(9):1043-9. doi: 10.1038/nm.3645. Epub 2014 Aug 17. PMID: 25129481; PMCID: PMC4362521.

14. Pratt CH, King LE Jr, Messenger AG, Christiano AM, Sundberg JP. Alopecia areata. Nat Rev Dis Primers. 2017 Mar 16;3:17011. doi: 10.1038/nrdp.2017.11. PMID: 28300084; PMCID: PMC5573125.
15. Alshahrani AA, Al-Tuwaijri R, Abuoliat ZA, Alyabsi M, AlJasser MI, Alkhodair R. Prevalence and Clinical Characteristics of Alopecia Areata at a Tertiary Care Center in Saudi Arabia. Dermatol Res Pract. 2020 Mar 13;2020:7194270. doi: 10.1155/2020/7194270. PMID: 32231700; PMCID: PMC7093899.
16. Schoenborn JR, Wilson CB. Regulation of interferon-gamma during innate and adaptive immune responses. Adv Immunol. 2007;96:41-101. doi: 10.1016/S0065-2776(07)96002-2. PMID: 17981204.
17. McElwee KJ, Gilhar A, Tobin DJ, Ramot Y, Sundberg JP, Nakamura M, Bertolini M, Inui S, Tokura Y, King LE Jr, Duque-Estrada B, Tosti A, Keren A, Itami S, Shoenfeld Y, Zlotogorski A, Paus R. What causes alopecia areata? Exp Dermatol. 2013 Sep;22(9):609-26. doi: 10.1111/exd.12209. PMID: 23947678; PMCID: PMC4094373.
18. Agamia N, Apalla Z, El Achy S, Abdelmaksoud E, Kandil N, Abozeid S. Interferon-gamma serum level and immunohistochemical expression of CD8 cells in tissue biopsies in patients with alopecia areata in correlation with trichoscopic findings. Dermatol Ther. 2020 Jul;33(4):e13718. doi: 10.1111/dth.13718. Epub 2020 Jul 5. PMID: 32472615.
19. Arca E, Muşabak U, Akar A, Erbil AH, Taştan HB. Interferon-gamma in alopecia areata. Eur J Dermatol. 2004 Jan-Feb;14(1):33-6. PMID: 14965793.
20. Kasumagic-Halilovic E, Prohic A, Karamehic J. Serum concentrations of interferon-gamma (IFN-g) in patients with alopecia areata: correlation with clinical type and duration of the disease. Med Arh. 2010;64(4):212-4. PMID: 21246917.
21. Lortkipanidze NT, Tevzadze MSh, Kamkamidze GK. [Interferon-gamma and neopterin in alopecia areata]. Georgian Med News. 2005 Jun;(123):53-7. Russian. PMID: 16052058.
22. Ataseven A, Saral Y, Godekmerdan A. Serum cytokine levels and anxiety and depression rates in patients with alopecia areata. Eurasian J Med. 2011 Aug;43(2):99-102. doi: 10.5152/eajm.2011.22. PMID: 25610172; PMCID: PMC4261352.
23. Tabara K, Kozłowska M, Jędrowiak A, Bienias W, Kaszuba A. Serum concentrations of selected proinflammatory cytokines in children with alopecia areata.

Postepy Dermatol Alergol. 2019 Feb;36(1):63-69. doi: 10.5114/ada.2019.82826. Epub 2019 Feb 22. PMID: 30858781; PMCID: PMC6409873.

24. Damsky W, King BA. JAK inhibitors in dermatology: The promise of a new drug class. J Am Acad Dermatol. 2017 Apr;76(4):736-744. doi: 10.1016/j.jaad.2016.12.005. Epub 2017 Jan 28. PMID: 28139263; PMCID: PMC6035868.
25. Kim WH, An HJ, Kim JY, Gwon MG, Gu H, Lee SJ, Park JY, Park KD, Han SM, Kim MK, Park KK. Apamin inhibits TNF-α- and IFN-γ-induced inflammatory cytokines and chemokines via suppressions of NF-κB signaling pathway and STAT in human keratinocytes. Pharmacol Rep. 2017 Oct;69(5):1030-1035. doi: 10.1016/j.pharep.2017.04.006. Epub 2017 Apr 18. PMID: 28958612.
26. Tabara K, Kozłowska M, Jędrowiak A, Bienias W, Kaszuba A. Serum concentrations of selected proinflammatory cytokines in children with alopecia areata. Postepy Dermatol Alergol. 2019 Feb;36(1):63-69. doi: 10.5114/ada.2019.82826. Epub 2019 Feb 22. PMID: 30858781; PMCID: PMC6409873.
27. Nestor MS, Ablon G, Gade A, Han H, Fischer DL. Treatment options for androgenetic alopecia: Efficacy, side effects, compliance, financial considerations, and ethics. J Cosmet Dermatol. 2021 Dec;20(12):3759-3781. doi: 10.1111/jocd.14537. Epub 2021 Nov 6. PMID: 34741573; PMCID: PMC9298335.