Evaluation of IL12 and IL-15 serum levels in alopecia areata patients in AL-Diwaniyah province

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Abstract

Background: Recent theories have shown that some immune variants found in patients can be associated with Alopecia, especial alopecia areata type.

Objective: the aim of current study is to Detection, Assessment, Estimation of some hematological parameters in alopecia areata (AA).

Material and methods: A retrospective- study was performed from July 2022 - February 2023. A total of 120 patients with alopecia to AL-Qadisiyah hospitals, private clinicals, AL-Karamah private hospitals in both sex and different age were studied. The serum was taken from the patients by drawing blood, and separating it with a centrifuge then applying immunological techniques on it.

Results: The results revealed that alopecia areata are 80(66.66%) sample , other types(the alopecia universalis and alopecia totalis) are 40(33.22%) sample, out of 120 samples. Through the results, immunological changes were found in patients with alopecia areata, the total WBC (8051.1 ± 376.22) , cell/mm3 ,as compared with the healthy control (7056.0 ± 249.6) cell/mm3. results of IgE 2.568±0.189 of patients and 0.043±0.022 , while Both IgG and IgM have an inverse correlation with the severity of the disease. level of interluiken 12 of pateints with alopecia areata 472.69 ± 11.53a, patients with Universalis and totalis 339.63 ± 23.76a, compared with control(n=60)122.40±16.68b, level of interluiken 15 with alopecia areata was the highest 532.54± 22.54a , however the pateints with unevirsalis and totalis 464.55±45.98a , compared with control 118.55±23.51b.The results of CD4 pateints with alopecia areata was the highest 4.06 ± 0.93 compared with control 3.18 ± 0.65 , The results of CD8 pateints with alopecia areata was the highest (n=80)323.7± 127.3 compared with control group(n=60) 159.8±62.1

Conclusion: the study highlighted, the presence of some immunological variables in patients that lead to alopecia and gives the basis for a larger and more complete study of immunological disorders in patients with alopecia areata or totalis.

Keywords: *Alopecia areata, Alopecia universalis, Alopecia totalis, immunological variables.*

INTRODUCTION

Alopecia mentions to a hair loss from either part ofthe head or body. The head is usually involved atleast(Kim et al. ,2022). Alopecia is one of the most commonprotests seen in the practice of dermatology. Sometimesthe definite diagnosis is difficult and active medicaltreatments are limited, though (non-cicatricial) alopeciais prevalent hair loss causes (Anudeep et al. ,2022). Alopecia areata (AA) is considered to be anautoimmune disease due to thumping T-cell responseagainst hair follicle-

auto-antigen, non-scarring, thepattern of alopecia that presents in areas circularly sharplydefined (Pinto et al. ,2019). distinguished bypatchy hair loss from the scalp as well as other parts ofthe body, without any inflammation (Park et al, 2020).The Scalp is the region most often affected. AA canoccur in any age and both sex (Perera et al, 2015). AA is divided according to the pattern plus severityof hair loss. All hair scalp hair loss is called A.totalis (AT),and loss of complete body hair is called A. universalis(AU), it can also affect eyebrows, nails, beard, and otherparts of the body ,besides scalp and body hair (Bhat et al, 2017; Beigi, 2018) and there are Clinical subtypescomprise, AA sisiapho, AA diffuse, AA reticularis, patchyAA ,and AA ophiasis (Strazzulla et al, 2018).

The exist etiopathogenesis of AA is not yet fullyunderstood, but genetics, infections, melanocyte defects,immunological factors, keratinocyte degeneration,neurological factors and emotional stress. Triggers areall considered possible contributors (Darwin et al, 2018).This disease is extremely correlated with the incidenceof psychical comorbidities (Aghaei et al, 2014).The first signal of autoimmunity in AA toward hair follicles involved a clustering of inflammatory cells swarmof bees in the direction of the hair follicles.

Bulb region (Strazzulla et al, 2018), the hair follicles auto- antigen, such as (protein associated with melanin)are also suggested to be recognized by cytotoxic T cells(Gilhar et al, 2016).Hair follicle is an immune-privileged structure. ThisImmune Privilege (IP) is not limited to the matrix onlybut also extends to the bulge region that protects the hair stem cells (Meyer et al, 2008). There are severalfeatures that contribute to the capacity of the hair bulb toescape the reactions of the immune system against(melanocyte peptides), and-or (self-keratinocyte) (Wanget al, 2016). Like down regulation of the receptors onthe NK cells as well as CD8+ cytotoxic T cells andCD4+Tcell (Lanier, 2015; Ebrahim et al, 2019).

Hematological parameters have been consideredbiomarkers of the diagnostic during several dermatologicaldiseases with the inflammatory method like psoriasis(Asahina et al, 2017). Cytokines and chemokine play avital role in the immune process of AA (Iorizzo and Tosti,2018; Kawen, 2019).

The infiltration of Th1 and Th2 cells producing a range of cytokines maybe observed in the hair follicle area., including interleukin(IL) 12 (IL-12), IL-15 in the blood serum of patients, which confirms that Th1 lymphocytes play a role in the etiopathogenesis of AA(Messenger, A. G. 2021). Alopecia areata is an example of an autoimmune disease of the hair follicle with a geneticbackground. Hair loss in alopecia areata is caused by lymphocytic infiltration around the hairfollicle. IgG antibodies against hair follicle cells are also found in people suffering from alopecia areata. Autoreactive CD8 +, CD4 +, NK and pDC cells infiltrate around the hair follicle in the growth phase (anagen). Increased activity of cytokines disrupts normal hair growth and terminates the anagen phase.

Aims of the study : To identify correlation between immunological, hematological factors and alopecia through measuring of the following; the role of serum cytokine level (IL-12 , IL-15,IgG,IgM and IgE) and find relationship between alopecia and this biomarkers.

Materials and Methods

Patients characterization

A total of 120 serum were obtained by clean needle after drawing 5 ml of blood from a vein

and placing it in the transport tube, then separate the serum by centrifuge and freeze it until useunder aspetic conditions during the study period from 1/7/2022 till 1/2/2023 from all age patients at(1-55) years old patients suffering from alopecia admitted to AL-Diwaniyah Teaching Hospital, AL-Karamah Hospital and private clinics.

Ethical Considerations

This study was approved by the Medical Ethics Committee at the Ministry of Health in Iraq.

Measurment of serum IgG, IgM, IgE, IL-12,IL-15 levels by ELIZA

A venous blood sample was collected (5ml) fromevery patients, as well as healthy control groups. Someof sample was put in gel tube and left to clot forapproximately 30 min. Then, serum was separated .afterthat, it was kept at -20ºC until use in analysis, the serumlevels of IL-17A was evaluated quantitatively usingEnzyme-Linked Immunesorbent Assay (ELISA)technique, ELISA tests had been performed inaccordance to the manufacture directions (Mybiosource,USA). The remains of the sample (whole blood) werecollected in an EDTA tube, for hematological assay usingauto-hematology device (Mindary, China) the opticaldensity was measured of each well by ELISA reader at450 nm and the IL12 , IL-15 ,IgM,IgG and IgE concentration results were calculated by interpolated from the standard curve.

Statistical analysis

The results are expressed by Pearson’s chi-square test and Fisher’s exact test were used to compare the risk factors. A p-value ＜0.05 was considered statistically significant.

Results

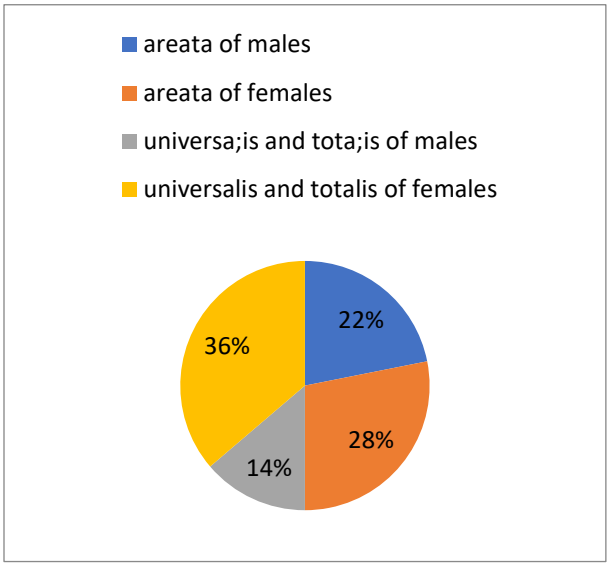
Patients characterization

120 A total number of patients with alopecia, 80 of whom were infected with alopecia areata (35 of males, 45 of females) and 40 were infected with alopecia totalis and universalis (11 of males, 29 of females) . As shown in table (1).

Table (1): the distribution of studied groups according to their gender

Groups	**Patients (n=120)**			
		Areata (n=80)	Universalis and totalis(n=40)	**probability**
Gender number percentage(%)	Males	35(43.75)	11(27.5)	**p>0.05**
	Females	45(56.25)	29(72.5)	

Figure(1): The number of alopecia areata, universalis and totalis of males and females



The current study indicated results showing the number of leukocytes (total and differential) according to disease severity, the current result indicated that the patient groups have elevated mean of the total WBC as compared with the healthy control, as shown in the table (2).

Table (2): Distribution of WBCs according to the studied groups

Groups		Patients(n=120)		Control (n=60)
		Areata (n=80)	Universalis and totalis(n=40)	
WBC scount (cell/mm3) (Mean ± SE)		376.22a±9031.1	395.22b±8371.5	**249.6b±6030.0**
WBCs Differential percentage(%)	Neutrophil	58.0	57.02	**58.64**
	Lymphocyte	33.93	32.66	**32.64**
	Monocyte	5.12	5.20	**5.26**
	Eosinophil	2.32	2.33	**1.67**
	Basophil	0.52	0.57	**0.51**

Duncan test: similar letters referred to a non-significant difference ($P > 0.05$), different letters referred to a significant difference ($P \leqslant 0.05$)

Table (3): results of immunoglobulin

Immunoglobulin (g/L)	Mean±SD(Patients)	Mean ±SD(control)
IgM	0.034±0.033	**0.012±0.083**
IgG	0.032±0.045	**0.011±0.103**
IgE	0.189±2.568	**0.022±0.043**

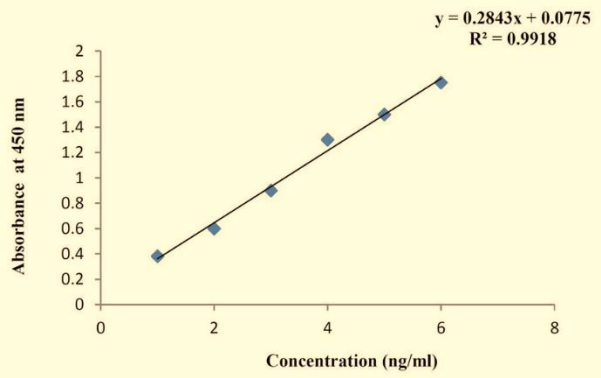
results of IgE 2.568±0.189 of patients and 0.043±0.022 from control that shown a clear difference between patients with alopecia areata and controls, and this means a significant difference of IgE in affected patients, as they have higher sensitivity than controls or normal people.

The results of interluiken 12 through the table(4) and curve, showed that the pateints with alopecia areata was the highest (n=80) 472.69±11.53a however the pateints with unevirsalis and totalis (n=40) 339.63±23.74a , compared with control 122.40±16.68b . The results of interluiken 15 the pateints with alopecia areata was the highest (n=80) 532.54± 22.54a , however the pateints with unevirsalis and totalis (n=40) 464.55±45.98a , compared with control 118.55±23.51b .

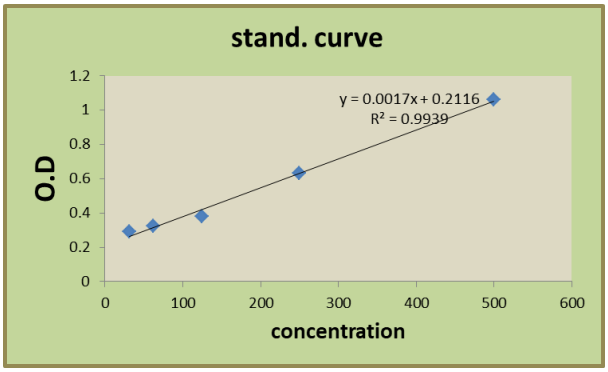
Table (4): Serum level Interleukin-12 , 15 and control

	Patients (n=120L)		
	Areata (n=80)	Universalis and totalis)(n=40	**Control (n=60)**
IL-12 level (pg/ml) (Mean ± SE)	11.53a±472.69	23.74a±339.63	**16.68b±122.40**
IL-15 level (pg/ml) (Mean ± SE)	22.54a±532.54	45.98a±464.55	**23.51b±118.55**

Duncan test: similar letters referred to a non-significant difference ($P > 0.05$), different letters referred to a significant difference ($P \leqslant 0.05$)



The standard curve of IL-12



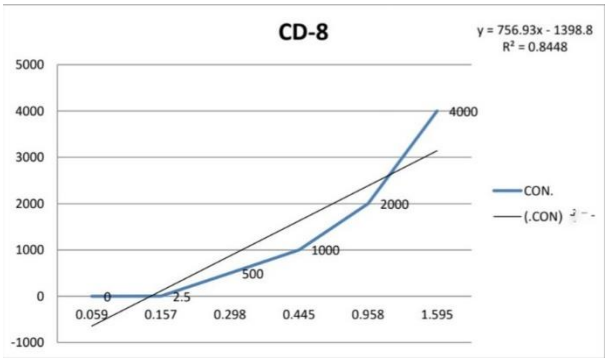
The standard curve of IL-15

The results of CD4 and CD8 through the table(5) and curve, showed that the pateints with alopecia areata was the highest (n=80) 4.06±0.93 of CD4 compared with control(n=60) 3.18±0.65, while the results of CD8 showed 323.7±127.3 compared with control 159.8±62.1

Table (5): Test variables' descriptive statistics for the groups under study

Variable	Patients group (n = 80) Mean±SD	Control group (n = 60) Mean±SD	P by t test
CD4	0.93±4.06	0.65±3.18	**0.01**
CD8	127.3±323.7	62.1±159.8	**0.03**

The standard curve of CD4



The standard curve of CD8

Discussion

The current study draws attention to the immunological conundrum related to the pathophysiology of AA. This was demonstrated by the significantly higher tissue and serum levels of IL-12, IL-15 , as well as CD4, CD8 in patients as compared to controls.

Knowing alopecia is an unspecific, chronic inflammatoric reaction, bound to follicles and maintained by cytokinemediated TH1-reactions. HLA class I molecules are expressed on virtually all nuclea-ted cells and platelets and present antigens to CD8 Tcells. HLA class II molecules have three main subclasses(DR, DQ, and DP); they are found on speci¢c immune cells,including B cells, activated T cells, macrophages, keratino-cytes, and dendritic cell and present peptides to CD4 T cells. Because class II molecules are associated

with antigen presenta-tion, many studies have focused on this area of the HLAmolecule. The association of AAwith HLA-DR and HLA-DQ antigenssuggests a role for CD4 Tcells in this disease, as MHC class IImolecules present peptides to CD4 cells. Recent transplanta-tion studies indicate that CD8 cells are also involved in AA,implicating MHC class I HLA-A,B,C molecules, which are asso-ciated with the presentation of peptides to CD8 Tcells, in ad-dition to MHC class II molecules. AA is considered to be an autoimmune diseasecaused by CD4 and CD8 T cells invading immune-privileged anagen-stage hair follicles causing a lossof tolerance. Both T helper 1 (Th1) and T helper2 (Th2) cytokine responses are involved with animalmodels of AA, which could explain the associationof AA with both antibody mediated. heassociation of AA with autoimmune disease is verystrong and is suported by the latest populationalstudies, Immune cells attack the skin in both AA and atopicdermatitis AA share a Th2 cytokinepattern and increased levels of IgE antibodies, mast cells,and eosinophils. Hair follicle specific IgG autoantibodies have beenfound in increased concentrations in the peripheral blood of AA affected individuals compared to‘‘normal’’, non-affected humans.

There is a Th2 (interleukin (IL) 12) response in localizedAA versus a Th1 (interferon (IFN) - gamma) responsein generalized AA.

peri- and intra-follicular inflammation, mostly made up of T cells, is how AA typically manifests. This observation, however, merely offers circumstantial proof of autoimmunity. The inflammatory cells may have developed as a subsequent reaction to an earlier stimulation, such as an infection or defective hair follicles.

Reference

Venten, I., Hess, N., Hirschmuller, A., Altmeyer, P., & Brockmeyer, N. (2006). Treatment of therapy-resistant Alopecia areata with fumaric acid esters. European journal of medical research, 11(7), 300.

Kageyama, R., Ito, T., Hanai, S., Morishita, N., Nakazawa, S., Fujiyama, T., ... & Tokura, Y. (2021). Immunological properties of atopic dermatitis-associated alopecia areata. International Journal of Molecular Sciences, 22(5), 2618.

Chen, D., Huang, W., Zhongjie, W., Feifeng, R., Luo, L., Jun, Z., ... & Tang, L. (2022). Good efficacy achieved by baricitinib in the treatment of anti-MDA5 antibody-positive dermatomyositis with alopecia areata. Rheumatology, 61(8), e221-e223.

McElwee, K. J., Spiers, E. M., & Oliver, R. F. (1996). In vivo depletion of CD8+ T cells restores hair growth in the DEBR model for alopecia areata. British Journal of Dermatology, 135(2), 211-217.

Ito, T., Kageyama, R., Nakazawa, S., & Honda, T. (2020). Understanding the significance of cytokines and chemokines in the pathogenesis of alopecia areata. Experimental Dermatology, 29(8), 726-732.

Agamia, N., Apalla, Z., El Achy, S., Abdelmaksoud, E., Kandil, N., & Abozeid, S. (2020). Interferon‐gamma serum level and immunohistochemical expression of CD8 cells in tissue biopsies in patients with alopecia areata in correlation with trichoscopic findings. Dermatologic Therapy, 33(4), e13718

Sorour, N. E., Musatafa, A. I., Abdel Sattar, R. H., & Lasheen, Y. F. (2020). Expression of Death Receptor 3 (DR3) on Circulating CD4 Positive Lymphocytes in Patients

with Alopecia Areata. Benha Journal of Applied Sciences, 5(6 part (1)), 157-160.

Aldaffaie, S. T. H., Hussein, T. A., & Muhieldden, A. A. R. (2022). Estimation of CD3, CD4, and CD8 in Iraqi patients with alopecia areata and alopecia universalis. Journal of Cosmetic Dermatology, 21(3), 1286-1291

Rinaldi, F., Trink, A., & Pinto, D. (2020). Efficacy of postbiotics in a PRP-like cosmetic product for the treatment of alopecia area Celsi: a randomized double-blinded parallel-group study. Dermatology and Therapy, 10(3), 483-493.

Manolache, L., & Benea, V. (2007). Stress in patients with alopecia areata and vitiligo. Journal of the European Academy of Dermatology and Venereology, 21(7), 921-928.

Werner, B., & Mulinari-Brenner, F. (2012). Clinical and histological challenge in the differential diagnosis of diffuse alopecia: female androgenetic alopecia, telogen effluvium and alopecia areata-part I. Anais brasileiros de dermatologia, 87, 742-747.

Wasserman, D., Guzman‐Sanchez, D. A., Scott, K., & McMichael, A. (2007). Alopecia areata. International journal of dermatology, 46(2), 121-131

Soliman, M. M., Abd El Gayed, E. M., Saleh, S. M., & Bazid, H. A. S. (2022). Assessment of serum level of interleukin-15 in patients with alopecia areata. Menoufia Medical Journal, 35(3), 1026.

Khafagy, N. H., El Din Zaki, M. S., & Hussein, A. M. (2021). Serum Interleukin-15 in Alopecia Areata and its correlation with dermoscopic findings. QJM: An International Journal of Medicine, 114(Supplement_1), hcab093-011.

Ebrahim, A. A., Salem, R. M., El Fallah, A. A., & Younis, E. T. (2019). Serum interleukin-15 is a marker of alopecia areata severity. International Journal of Trichology, 11(1), 26

Aldzhabali, M. A. M., & Kuts, L. V. (2022). Serum levels of IL-2 and IL-17A are related to clinical type and severity of alopecia areata.

Hatif, S. T., Hussein, T. A., & Muhieldden, A. A. R. (2020). Evaluation of il17-a serum levels in iraqi patients with alopecia areata and alopecia universalis. Biochem. Cell Arch, 20, 6419-6425

Polak‐Witka, K., Rudnicka, L., Blume‐Peytavi, U., & Vogt, A. (2020). The role of the microbiome in scalp hair follicle biology and disease. Experimental Dermatology, 29(3), 286-294

Rinaldi, F., Trink, A., Papale, A., Giuliani, G., & Pinto, D. (2022). Clinical Translation of Microbiome Research in Alopecia Areata: A New Perspective?. Cosmetics, 9(3), 55

Barquero-Orias, D., Moreno-Arrones, O. M., & Vañó-Galván, S. (2021). Alopecia and the microbiome: a future therapeutic target?. Actas Dermo-Sifiliográficas (English Edition), 112(6), 495-502..

Haidamak, J., Dos Santos, G. D., de Souza Lima, B. J. F., Soares, V. M., de Menezes, R. V., Bisson, A. A., ... & do Rocio Klisiowicz, D. (2019). Scalp microbiota alterations in children with pediculosis. Infection, Genetics and Evolution, 73, 322-331...

Basmanav, F. B., & Betz, R. C. (2022). Translational impact of omics studies in alopecia areata: recent advances and future perspectives. Expert Review of Clinical Immunology, 18(8), 845-857