Al-Azhar Med. J. (Medicine). *Vol. 52(3), July, 2023, 803 - 820*
DOI: 10.21608/amj.2023.315119
https://amj.journals.ekb.eg/article_315119.html

ASSESSMENT OF SERUM LEVELS OF ANTIGLIADIN ANTIBODIES (IgG and IgA) IN PATIENTS WITH ALOPECIA AREATA AND THEIR RELATION TO SEVERITY OF THE DISEASE

By

***Alaa Makram Mohamed, *Hanan Mohamed Ali Darwish, *Hoda Kamal Al-Sebaay, **Sarah younes Abd El-Aziz**

*Dermatology and Venereology Department, Faculty of Medicine for girls, Al-Azhar University

**Clinical Pathology Department, Faculty of Medicine for girls, Al-Azhar University

Corresponding Author: Alaa Makram Mohamed

Mobile No.: (+2) 01098045467, **E-mail:** dr.alaa.makram01@gmail.com

ABSTRACT

Background: Alopecia areata is an autoimmune disease mediated by T-lymphocytes in which autoantigens play an important part in activating T-cells and is associated with other autoimmune diseases. Hair follicle is an immune-privileged site where major histocompatibility complex (MHC) Class I and II molecules are not expressed. Celiac disease (CD) is an autoimmune disorder that affects people who are genetically predisposed to develope an immune reaction to gluten. The small intestine is the primary site of CD.The clinical presentation, on the other hand, can be distinguished by both intestinal and extra-intestinal manifestations. Serological tests are initially recommended for the diagnosis of CD. One of the most sensitive and specific tests is anti-tissue transglutaminase IgA. Other serological tests include anti-gliadin IgA and IgG.

Aim of the Work: To evaluate serum level of antigliadin antibodies (Ig A and Ig G classes) in patients with alopecia areata and their relation to the severity of the disease.

Patients and Methods: This case control study included 60 participants including 30 patients with alopecia areata and 30 healthy individuals as controls. Patients were selected from Dermatology outpatient clinic in AL Zahraa University hospital during the period from November 2020 to April 2021.

Results: Out of 30 patients with alopecia areata included in the study the severity of alopecia tool score (SALT) score was S5 in 8 (26.7%) patients, S4 in 2 (6.7%), S3 in 1 (3.3%), S2 in 5 (16.7%) and S1 in 14 (46.7%). Antigliadin Ig A was only positive in 8 (26.7%) S5 patients while IgG was positive in all 30 patients with 2 positive cases in control group resembling (6.7%). There was statistically significant difference between control and patients groups regarding IgA and IgG levels, as higher values was found in patients than controls,the median (IQR) of Ig A in patients was 9.11 (6.88 – 12.2) while in control group was 1.56 (0.97 – 3.29)with p-value < 0.001. By ROC curve for sensitivity and specificity of IgA and IgG, we found that there were 100% sensitivity and specificity of IgA with cutoff point >4.25 and also for IgG with cutoff point >13.

Conclusion: There was a high statistically significant association between antigliadin antibodies (Ig A and Ig G class), and AA and its severity, with100% sensitivity and specificity of IgA with cutoff point >11.56 and also for IgG with cutoff point >41.8. An active search for CD using serological screening tests has been recommended to diagnose the numerous cases of subclinical CD in AA patients.

Keywords: antigliadin antibodies, (IgG and IgA), alopecia areata.

INTRODUCTION

Alopecia areata (AA) is a common form of immune-mediated alopecia in which an autoimmune attack on the proximal hair follicle results in non-scarring hair loss ranging in presentation from circular patches on the scalp to total scalp or full body hair loss *(Elana Putterman, et al., 2019)*.

Alopecia areata typically begins as a small, well-demarcated patch of hair loss on the scalp or beard that may spontaneously resolve without treatment. In about 30% of cases, the disease relapses; in some cases, hair loss may progress and affects the entire scalp (alopecia totalis) or the entire body (alopecia universalis) *(Breitkopf T, et al., 2013)*.

Studies suggest that alopecia areata prevalence in the USA has remained reasonably constant over the past 50 years. It is similar to the upper limit estimates in the 1970s at approximately 0.21% and with "moderate to severe" disease estimated at 0.09% and possible lifetime prevalence as high as 2.51 % *(Michael Benigno et al., 2020)*.

Reports showed that there is no gender predominance in alopecia areata, it is considered as a disease of all age groups; however, most patients present at age of 21–40 years *(Richard, et al., 2019)*.

In some studies, alopecia areata is reported accompanied with other autoimmune illnesses such as Hashimoto's thyroiditis, vitiligo, diabetes, lichen planus, and also in a few reports, celiac disease (CD) (gluten-sensitive enteropathy) *(El Gayyar, et al., 2011)*.

Celiac disease (CD) is a chronic autoimmune systemic disease associated with an enteropathy triggered by gluten intake which affects genetically predisposed individuals of both sexes and can develop at any age. Gluten and its major protein fractions, gliadin and glutenin, are present in wheat, rye, barley, oats, related species and hybrids, and processed foods *(Bai, et al., 2013)*.

Celiac disease patients are more frequently affected by other immune-mediated disorders (ID) compared to the general population, as reported in previous studies, mainly thyroid and skin diseases. This observation may be partially explained by a possible spread of the adaptive immune response, initially triggered in the gastrointestinal tract, to other tissues. Hashimoto"s thyroiditis is the most frequently associated ID, followed by several skin disorders, such as psoriasis, atopic dermatitis (AD), vitiligo, systemic lupus erythematosus (SLE), alopecia areata (AA), and oral lichen planus (OLP) *(Elli, et al., 2012)*.

Increased prevalence of serum markers for celiac disease has been reported among patients with skin disorders like psoriasis *(Kolchak, et al., 2018)*.

The finding that intestinal intraepithelial cytotoxic CD8 Tlymphocytes (IE-CTLs) can kill epithelial cell, based on recognition of inflammatory and stress signals provides an explanation of how IECTL, without being gluten specific, can play a major role in celiac disease pathogenesis and in particular in epithelial cell destruction *(Jabri, Abadie V, 2015)*.

Similar to the anterior chamber of the eye, testes, brain, adrenal cortex and the

fetomaternal placental unit, the hair follicle is a site of relative immune privilege (IP), a term that was initially used to describe an environment that can tolerate allografts without rejection by the host immune system. It has been suggested that immune mediated alopecias such as alopecia areata result from an insult to hair follicle immune privilege, which causes inflammatory agents to infiltrate and attack the hair follicle *(Kinori, et al., 2011)*.

Substantial progress in basic and clinical immunology research suggests that alopecia areata is a CD8+ cell, Th1-type autoimmune reaction against anagen stage hair follicles *(McElwee, et al., 2013)*.

Alopecia areata is a skin disease and can be easily diagnosed, but can be a manifestation of an internal disease such as celiac, so celiac disease can be treated before any complications *(Mokhtari, et al., 2016)*.

Antibodies to gliadin have, for some years, been the first serological step in the diagnosis of celiac disease. These antibodies in both the IgG and IgA classes were detectable with immunoassay methods. These tests show better diagnostic accuracy in the IgA class, and were considered very useful in children because they were the first and often the only marker present *(Rubio-Tapia, et al., 2013)*.

Several studies have identified pediatric patients with celiac disease who were found to have positive antigliadin antibody and negative tissue transglutaminase or antiendomysial antibodies, suggesting antigliadin antibody may still be appropriate when screening this population *(Karnsakul, et al., 2012)*.

It has recently been observed that about half of patients suffering from non-celiac gluten sensitivity do have high-IgG antigliadin antibody and this remains, to date, the only laboratory marker for this disease *(Anna, et al., 2012)*.

Prevalence of antigliadin IgA antibody is significant among patients with psoriasis not diagnosed with celiac disease or non-celiac gluten sensitivity *(Kolchak, et al., 2018)*.

According to most of the reports, hair regrew in patients with alopecia areata and celiac disease after they accepted a gluten-free diet *(Mokhtari, et al., 2016)*.

The severity of alopecia tool score (SALT) score was introduced as a mathematical approach to the determination of hair loss and hair regrowth. Briefly, the % of scalp hair loss in each of the sides, back and top of the scalp were determined independently, each was multiplied by the % scalp covered in that area of the scalp and the products of each section summed for a final total % hair loss, designated as the Severity Alopecia Tool or SALT score *(Olsen, 2011)*.

AIM OF THE WORK

To evaluate serum level of antigliadin antibodies (Ig A and Ig G classes) in patients with alopecia areata and their relation to the severity of the disease.

PATIENT AND METHODS

This case control study included 60 participants including 30 patients with alopecia areata and 30 healthy individuals as controls. Patients were selected from

Dermatology outpatient clinic in AL Zahraa University hospital during the period from November 2020 to April 2021.

Patients: This group included 30 patients with alopecia areata, the patients were selected according to the following inclusion and exclusion criteria.

Inclusion criteria: Patients diagnosed clinically as alopecia areata, Patients with alopecia totalis or universalis, participants of any age and participants of both sexes.

Exclusion criteria: Patients with previously diagnosed celiac disease, patients who were treated with a gluten-free diet, patients with history of chronic lower gastrointestinal symptoms, patients with history of autoimmune disorders as psoriasis, dermatitis herpitiform, vitiligo, alopecia areata, Sjogren's syndrome or rheumatoid arthritis, pregnant women, nursing mothers and people who did not cooperate with the investigation.

Control group: Control samples for antigliadin antibodies were obtained from 30 healthy individuals matched for the criteria.

Methods: The study purpose was to assess serum levels of antigliadin antibodies (IgA and IgG) in patients group compared to control group.

Ethical considerations: This study was approved by the local ethical committee of AlAzhar Faculty of Medicine for Girls. Participation in this study was not obligatory. The included cases were clarified about the aim of the study, a written informed consent was obtained from participants in this study or their parents and/or caregivers prior to participation in the study.

Complete history taking including: Personal history of age, sex, residence, occupation, marital status, special habits and food habits. Present history including onset, course and duration of disease, history of treatment and recurrence. Medical history of associated disorders, autoimmune diseases, medications (as chemotherapy, gold, colchicine, anticoagulant, anticonvulsant, antidepressant),surgical procedures and history regarding bowel habits.

Examination including: General examination for associated systemic diseases and Dermatological examination: Skin examination to diagnose alopecia areata and its clinical variants such as patchy, ophiasis, totalis, universalis, Dermoscopic examination using non-contact polarized dermoscopy (DermLite HUD dermoscope, 3Gen, USA) (Fig. 29), connected to samsung galaxy S9 plus dual lens 12 mega pixels digital camera (model SM-G965F/DS) and In the current study, dermoscopic examination was used to diagnose AA according to the criteria defined by Mane et al., who said that dermoscopy allows diagnosis of AA by detection of cadaverized hairs (black dots), exclamation mark hairs (tapering hairs), broken hairs, yellow dots and clustered short vellus hairs in the hair loss areas (Mane et al., 2011).

Clinical assessment of the degree of AA: The extent of scalp hair loss was determined according to the severity of alopecia SALT score (Olsen et al., 2004) as follows; Dividing the scalp into 4 quadrants, visually determining the percentage of scalp hair loss in each quadrant and adding the numbers together with a maximum score of 100% and

Assessment of scalp hair loss (SALT score), Body hair loss (B) and Nail (N) involvement as follows:

S = Scalp hair loss, S0 = no hair loss, S1 = <25% hair loss, S2 = 25-49% hair loss, S3 = 50-74% hair loss, S4 = 75-99% hair loss, S4a = 75-95% hair loss, S4b = 96-99% hair loss, S5 = 100% hair loss, B = Body hair loss, B0 = No body hair loss, B1 = Some body hair loss, B2 = 100% body hair loss (excluding scalp), N = Nail involvement, N0 = No nail involvement, N1 = Some nail involvement, N1a= Twenty nail dystrophy (must be all 20 nails).

Laboratory investigations: Collection of blood samples: Five ml of venous blood were withdrawn from each subject. The blood was collected in plain tubes and left at room temperature to clot. Serum was separated by centrifugation for 10 min at 1500 rpm. Serum was divided into 2 portions and put in Eppendorf tube for antigliadin antibodies (IgA and IgG) assay. Eppendorf tubes were stored at -20º C till time of assay. Assessment of serum antigliadin antibodies (IgA and IgG): Antigliadin antibodies (IgA and IgG) were assessed quantitatively in patients and controls by ELISA method with kits (ORG 534A and ORG 534G respectively, ORGENTEC Diagnostica GmbH, Mainz, Germany) and Positivity for both was considered ≥ 12 U/ml as provided by the manufacturer.

Statistical Analysis: Data were collected, revised, coded and entered to the Statistical Package for Social Science (IBM SPSS) version 23. The quantitative data were presented as mean, standard deviations and ranges when parametric and median, inter-quartile range (IQR) when data found non-parametric. Also, qualitative variables were presented as number and percentages.

The comparison between groups regarding qualitative data was done by using Chi-square test and/or Fisher exact test when the expected count in any cell found less than 5.

The comparison between two groups regarding quantitative data and parametric distribution was done by using independent t-test while with non-parametric distribution was done by using Mann-Whitney test.

The comparison between more than two groups regarding quantitative data and non-parametric distribution was done by using Kruskall-Wallis test.

Spearman correlation coefficients were used to assess the correlation between two quantitative parameters in the same group.

Receiver operating characteristic curve (ROC) was used to assess the best cut off point with its sensitivity, specificity, positive predictive value, negative predictive value and area under curve (AUC) of the studied marker.

The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the p-value was considered significant as the following:

P-value > 0.05: Non-significant (NS), P-value < 0.05: Significant (S), P-value < 0.01: Highly significant (HS).

RESULTS

Patients were 13(43.3%) males and 17 (56.7%) females. Their ages ranged from 2 – 47 years (Median (IQR) =17 (7 – 33) Y). Among those 30 patients; 17 (56.7%) were students, 5 patients (16.7%) were manual workers, 3 (10.0%) were employees, 4 (13.3%) were house wifes and 1 (3.3%) was nurse. 27 patients were smokers representing (90.0%).

As regard past history of studied patients, 4 patients representing (13.3%) had positive medical history and 26 patients representing (86.7%) had negative medical history. Only 2 patients representing (6.7%) had positive family history of AA. 2 patients representing (6.7%) had history of associated diseases **(Table 1)**.

Table (1): Descriptive for demographic data of the studied patients

		Patients group
		No. = 30
Age	Median (IQR)	17 (7 – 33)
	Range	2 – 47
Sex	Female	17 (56.7%)
	Male	13 (43.3%)
Occupation	Student	17 (56.7%)
	Employee	3 (10.0%)
	Worker	5 (16.7%)
	Housewife	4 (13.3%)
	Nurse	1 (3.3%)
Smoking	Negative	27 (90.0%)
	Positive	3 (10.0%)
Medical history	Negative	26 (86.7%)
	Positive	4 (13.3%)
Family history	Negative	28 (93.3%)
	Positive	2 (6.7%)
Associated diseases	Negative	28 (93.3%)
	Positive	2 (6.7%)

There were 6 patients (20.0%) of alopecia areata universalis, 2 patients (6.7%) of alopecia areata totalis, 6 patients with diffuse AA (20.0%) and 16 patients (53.3%) of localized AA.

In our study, the onset of AA was acute in 25 patients representing (83.3%), sudden in 3 patients representing (10.0%) and gradual in 2 patients representing (6.7%). The course was progressive in 16 patients representing (53.3%), stationary in 10 patients representing (33.3%) and remittent in 4 patients representing (13.3%). The duration of the disease ranged between 0.1 – 50 months with Median (IQR) 5 (3 – 18) months.

As regard nail changes 18 patients had nail changes representing (60.0%), 8 had longitudinal ridges representing (26.7%), 3 had pitting and longitudinal ridges (10.0%) and 1 had pitting (3.3%) **(Table 2)**.

Table (2): Descriptive for onset, coarse, duration, nail changes and SALT score for the studied patients.

		Patients group
		No. = 30
Onset	Acute	25 (83.3%)
	Sudden	3 (10.0%)
	Gradual	2 (6.7%)
Coarse	Progressive	16 (53.3%)
	Stationary	10 (33.3%)
	R&E	4 (13.3%)
Duration in months	Median (IQR)	5 (3 – 18)
	Range	0.1 – 50
Pattern of AA	Universalis	6 (20.0%)
	Diffuse	6 (20.0%)
	Localized	16 (53.3%)
	Totalis	2 (6.7%)
Nail changes	NAD	18 (60.0%)
	LR	8 (26.7%)
	Pitting	1 (3.3%)
	LR & pitting	3 (10.0%)
SALT	S1	14 (46.7%)
	S2	5 (16.7%)
	S3	1 (3.3%)
	S4	2 (6.7%)
	S5	8 (26.7%)

There was statistically significant difference between control and patients' group regarding IgA and IgG levels higher in patients than control, the median (IQR) in patients was 9.11 (6.88 – 12.2) while in control group was 1.56 (0.97 – 3.29) with p-value < 0.001.

And also, there was statistically significant difference between two groups according to positivity of IgA, with 8 positive cases in patients group representing (26.7%), also there was statistically significant difference according to IgG positivity with 30 positives in patients group resembling (100.0%) and 2 positive cases in control group resembling (6.7%) with p-value < 0.001 **(Table 3)**.

Table (3): Comparison between control and patients groups according to Ig A and Ig G levels and positivity

		Patients group	Control group	Test value	P-value	Sig.
		No. = 30	No. = 30			
IgA (U/ml)	Median (IQR) Range	9.11 (6.88 – 12.2) 4.96 – 31.8	1.56 (0.97 – 3.29) 0.16 – 4.25	6.653≠	0.000	HS
IgA (U/ml) positivity	Negative Positive	22 (73.3%) 8 (26.7%)	30 (100.0%) 0 (0.0%)	F	0.005	HS
IgG (U/ml)	Median (IQR) Range	28.45 (22.9– 43.4) 14.8 – 88.9	6 (3.06 – 8.53) 0.79 – 13	6.653≠	0.000	HS
IgG (U/ml) positivity	Negative Positive	0 (0.0%) 30 (100.0%)	28 (93.3%) 2 (6.7%)	F	0.000	HS

P-value > 0.05: Non significant; P-value < 0.05: Significant; P-value < 0.01: Highly significant
F: Fisher's Exact test; ≠: Mann-Whitney test

According to ROC curve there were 100% sensitivity and specifity of IgA with cutoff point >4.25 and also for IgG with cutoff point >13 **(Table 4)**.

Table (4): Sensitivity and specifity of IgA andIgG

Variables	Cut off point	AUC	Sensitivity	Specificity	+PV	-PV
IgA (U/ml)	>4.25	1.000	100.00	100.00	100.0	100.0
IgG (U/ml)	>13	1.000	100.00	100.00	100.0	100.0

There was statistically significant difference between the two groups of SALT score and IgA, IgG levels with P-value < 0.01 **(Table 5)**.

Table (5): Relation between SALT score and IgA, IgG levels

		SALT		Test value	P-value	Sig.
		Mild to moderateS1-4	Severe S5			
		No. = 22	No. = 8			
IgA (U/ml)	Median (IQR)	7.9 (6.3 – 9.96)	20.05 (15.95 – 25.65)	-4.127≠	0.000	HS
	Range	4.96 – 11.56	12.2 – 31.8			
IgG (U/ml)	Median (IQR)	25.55 (19.4 – 29.88)	61.3 (47.4 – 73.45)	-4.127≠	0.000	HS
	Range	14.8 – 41.8	43.4 – 88.9			

P-value > 0.05: Non significant; P-value < 0.05: Significant; P-value < 0.01: Highly significant
≠: Mann-Whitney test

DISCUSSION

Alopecia areata (AA) is a common form of non-scarring alopecia characterized by hair loss with no clinical signs of inflammation *(Bhat et al., 2017)*. The prevalence rate of the disease is approximately 1 in 1000 people worldwide. in range from 0.57% to 3.8% *(Villasante and Miteva et al., 2015)*.

AA is an autoimmune disease mediated by T-lymphocytes in which autoantigens play an important part in activating T-cells and is associated with other autoimmune diseases. Hair follicle is an immune-privileged site where major histocompatibility complex (MHC) Class I and II molecules are not expressed *(Bhat et al., 2017)*.

Celiac disease (CD) is an autoimmune disorder that occurs in genetically predisposed subjects who develope an immune reaction to gluten. CD primarily involves the small intestine. However, the clinical presentation can be characterized by both intestinal and extra-intestinal manifestations. *(Leonard et al., 2017)* The incidence of CD is up to 1% in the majority of populations. Genetic factors play an important role in the pathogenesis of CD *(Lebwohl et al., 2018)*.

The presentation of CD has shifted from the historically classic malabsorption pediatric symptoms in childhood to non-specific symptoms, which may be present also in adulthood. The symptoms classically include weight loss, chronic diarrhea, and failure to thrive. Non-specific symptoms are more common and include gastrointestinal manifestations as well as extra-intestinal manifestations, as osteoporosis, headache, iron deficiency, and chronic fatigue *(Hujoel et al., 2019)*.

For the diagnosis of CD, initially, serological tests are recommended. Anti-

tissue transglutaminase IgA is one of the most sensitive and specific tests. Anti-gliadin IgA and IgG are other serological tests. Other diagnostic procedures include endoscopy, biopsy of the small intestine, oral tolerance tests (xylose and lactulose), and radiographic studies of small intestine *(Mokhtari et al., 2016)*.

In the last years, skin diseases as alopecia areata are acquiring more and more importance among the extra-intestinal manifestations of CD *(Hujoel et al., 2019)*.

In this study we aimed to evaluate serum level of antigliadin antibodies (Ig A and Ig G class) in patients with alopecia areata and its relation to the severity of the disease.

We enrolled 60 subjects and divided them into 2 groups: Group 1: This group included 30 apparently healthy individuals as a control group and Group 2: This group included 30 patients with alopecia areata.

The Alopecia areata can occur at any age and the lifetime incidence appears to increase at an almost linear rate. The median age at diagnosis is 33. *(Mirzoyev et al., 2014)* in the current study the age ranged from 2 – 47 years with Median (IQR) =17 (7 – 33) Y).

In line with *Gilhar et al., 2012* study, as more than 66% of the patients were younger than 30 years old and only 20% were over 40 years old. (Kim et al., 2020) mentioned that up to 85.5% of patients' experience disease onset before the age of 40 years. Late-onset AA is relatively rare. And *(Seirafi et al., 2013)* who mentioned the AA disease was more common in younger age and under 20 years.

It can be explained through the fact that children's clinics were separated from adult's clinics. In this study 56.7% of AA patients were females and 43.3% were males.

Studies in the literature with regard to gender predominance in AA are conflicting. A systematic review concluded that there is no difference in the incidence of AA between males and females *(Villasante and Miteva, 2015)* and no sex predilection for AA was found in *(Bhat et al., 2017)* study. Although some studies show male preponderance. as *(Mokhtari et al., 2016)* study as the male represent 58.8%, and *(El Aziz Ragab et al., 2020)* (65%) were males.

While other studies showed female predominance as ours and *(Lundin et al., 2014)* study who explained that as it may be due to higher female concern regarding hair loss and subsequent treatment, and Male patients may be more likely to be diagnosed in childhood, while females are more likely to present in adolescence and have greater concomitant nail involvement or concomitant autoimmune diseases *(Lundin et al., 2014)*.

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 *(Jabbari et al., 2016)*. In the current study, 53.3% of patients had localized AA, followed by AAU and diffuse AA each of them represents 20% and 6.7%) had AAT.

In accordance with many studies found the patchy scalp being the most predominant type in *Alshahrani et al. (2020)*; *Fricke and Miteva (2015)* study and *Hallaji et al. (2011)* study.

Also, as *Mokhtari et al. (2016)* who found the most frequent type of AA was patchy (88.6%). The second frequent form was totalis (6.8%).

Nail involvement has been reported from 7% to 66%, with an average prevalence of approximately 30%, and is observed more frequently in children (>40%) than in adults (<20%), and more frequently in severe disease. It consists of nail pitting, or the typical "sandpapered" appearance of trachyonychia, a term originally proposed by Samman for the condition of a rough, lustreless nail plate. Less frequent nail changes are punctate leukonychia, spotted or red lunulae, transverse grooving of the nail plate (Beau's lines) and very rarely proximal separation of the nail plate from the matrix with shedding of nails (onychomadesis) *(Chelidze and Lipner, 2018)*.

As regard nail changes in this study, 60.0% patients had no nail changes, 26.7% had longitudinal ridges 10.0% had pitting and3.3% had both pitting and longitudinal ridges. while concerning the nail involvement, in *Mokhtari et al. (2016)* study, 88.5% of the patients had no involvement, but 5.7%, 2.9%, and 2.9% of them had pitting, ridging, and onycholysis, respectively.

On other hand Nail affection was not detected in association with alopecia areata patients in *El Aziz Ragab et al. (2020)* study.

The discrepancy may contribute to difference in participants characters as onset, severity and course of the disease.

In the present study, only 2 patients representing (6.7%) had positive family history of AA. In line with *Alshahrani et al. (2020)* who found positive family history of AA was positive in 6% of patients and El Aziz Ragab et al. (2020) whereas a positive family history of alopecia areata in first-degree relatives was detected in (5%) patients.

Also, *Mokhtari et al. (2016)* study concluded that most of the participants in the case group did not have any positive familial history of AA (82.9%).

Family history of AA in the three age subgroups (childhood, Adult-onset and Late-onset) in *Lyakhovitsky et al. (2019)* study ranged between 11% and 20%; however, the difference was not statistically significant.

Other Previous studies have shown that 3%–42% of AA patients report a family history of the disease, with a greater prevalence in those with early-onset and very low or no prevalence in those with late-onset AA *(Wu et al., 2013; Lee et al., 2014; Jang et al., 2015; Lyakhovitsky et al., 2017)*.

There were 2 patients representing (6.7%) in our study had history of associated diseases.

The association of AA with other autoimmune disease had variable results in the literature. Some studies showed that AA is not related to other autoimmune diseases *(Alkhalifah et al., 2010)*. However, many other studies demonstrated that AA is associated with several autoimmune diseases *(Abd El-Mawla and Maghrabi, 2015; Dainichi and Kabashima, 2017)*.

In *Alshahrani et al. (2020)* study, the 3 most commonly reported comorbidities

were hypothyroidism, asthma, and diabetes mellitus.

The association between AA and other diseases may contribute to a same auto immune background of both diseases.

In our study; The extent of the scalp involvement was evaluated using the Severity Alopecia Tool (SALT), as follows: S1 (<25% scalp involvement), S2 (26%~50%), S3 (51%~75%), S4 (76%~99%), and S5 (100%, alopecia totalis)and we found 46% were S1, 26.7% were S2, 16.7%, followed by S4(6.7%) and S3(3.3%), with statistically significant difference between SALT score and the duration of the disease with P-value < 0.01.and non-significant difference was found regarding other parameters as age or sex.

In line with *You and Kim (2017)* who found highly statistically significant difference with duration of the disease as higher duration related with sever disease, but differ with them regarding the sex as they found male sex was more common in the severe group (p=0.001). while female patients were more likely to have severe disease in a report from Singapore *(Tan et al., 2002)*. The association of sex with AA severity is unclear.

Both in the AA and in the CD, the presence of organ-specific autoantibodies has been demonstrated, with infiltration of T lymphocytes on the lesion site *(Pratt et al., 2017)*. In 1995, *Corazza et al. (1995)*, described for the first time an association between them. They described six patients where the diagnosis of AA preceded that of CD, and in at least four of them, the diagnosis of CD was made because of the presence of AA *(Corazza et al., 1995)*.

Since then, there have been other reports of this novel association. The study by *Ertekin et al. (2014)* found that the prevalence of CD in children with AA was 41.7% while in healthy children in the same city was 0.87%. This indicated that the prevalence of silent CD was very high among children with AA. Hence, it can be said that AA could be the only clinical manifestation of CD in some cases *(Ertekin et al., 2014)*.

A few case reports proposed an association between CD and AA. In a prospective trial of 256 AA patients, 6 had positive tissue transglutaminase and endomysial with positive biopsy *(Denham and Hill, 2013)*.

In another study, *Hallaji et al. (2011)* estimated the prevalence of anti-gliadin antibodies in patients with AA to be about 18% *(Hallaji et al., 2011)*.

In the current study, there was statistically significant difference between control and patients' group regarding IgA and IgG levels, as higher values were found in patients than control, the median (IQR) of Ig A in patients was 9.11 (6.88 – 12.2) while in control group was 1.56 (0.97 – 3.29with p-value < 0.001. With 8 positive cases in patients group representing (26.7%), and according to IgG positivity with 30 positives in patients group resembling (100.0%) and 2 positive cases in control group resembling (6.7%).

On other side, results of *Mokhtari et al. (2016)* study showed that in their population, there was no significant difference between the two groups in terms of celiac autoantibodies (anti-gliadin IgA, anti-gliadin IgG, and anti-tissue transglutaminase IgA), which could

be due to different demographic compositions.

In our study, we used ROC curve for sensitivity and specificity of IgA and IgG and found that there were 100% sensitivity and specificity of IgA with cutoff point >4.25 and also for IgG with cutoff point >13. Also, ROC curve for IgA and IgG was used to predict the class of SALT score and we found that there were 100% sensitivity and specificity of IgA with cutoff point >11.56 and also for IgG with cutoff point >41.8.

Regarding severity score, there was statistically significant difference between SALT score, pattern, and course of alopecia areata and also with IgA and IgG levels (P-value < 0.01). As higher values found in higher severity score.

Also, we found a positive correlation between IgA and IgG with the duration with p-value < 0.001.

In a recent case report by *(Aggarwal et al., 2020)*, they found an association in a 4½-year-old boy, who was referred by a dermatologist for gastrointestinal (GI) symptoms, as he complaint with loss of hair on the anterior half of his scalp for the past 8 months without any improvement. A diagnosis of CD was confirmed by duodenal biopsy and serology for antiendomysial and antigliadin antibodies. And treated with gluten-free diet (GFD), after 6 months later according to his parents, hair regrowth started once his GI symptoms subsided after the initiation of GFD.

Although remission and recurrence may be observed during the clinical course of AA, many patients on GFD have shown complete regrowth of hair with no further recurrence of AA at follow-up, these positive effects of GFD have been attributed to the normalization of the immune response *(Rodrigo et al., 2018)*.

All these finding detect high association between antigliadin antibodies (Ig A and Ig G class), CD and AA and its severity that needed further studies in multicenter.

CONCLUSION

There was a high statistically significant association between antigliadin antibodies (Ig A and Ig G class), and AA and its severity, with100% sensitivity and specificity of IgA with cutoff point >11.56 and also for IgG with cutoff point >41.8. An active search for CD using serological screening tests has been recommended to diagnose the numerous cases of subclinical CD in AA patients.

REFERENCES

1. **Abd El-Mawla AM, Maghrabi IA (2015):** Prevalence and treatment of Alopecia areata in Taif area, KSA. Saudi J Health Sci. (4):125-8.
2. **Aggarwal S, Aggarwal S and Kalra RK (2020):** Underlying celiac disease in children with alopecia areata: An association. Indian Journal of Paediatric Dermatology, 21(1), 77.
3. **Alkhalifah A, Alsantali A, Wang E, McElwee KJ and Shapiro J (2010):** Alopecia areata update: part I. Clinical picture, histopathology, and pathogenesis. Journal of the American Academy of Dermatology, 62(2), 177-188.
4. **Alshahrani AA, Al-Tuwaijri R, Abuoliat ZA, Alyabsi M, AlJasser MI and Alkhodair R (2020):** Prevalence and clinical characteristics of alopecia areata at a tertiary care center in Saudi Arabia. Dermatology research and practice, 2020.

5. **Bai JC, Fried M, Corazza GR, Schuppan D, Farthing M, Catassi C and LeMair A (2013):** World Gastroenterology Organisation global guidelines on celiac disease. Journal of clinical gastroenterology, 47(2), 121-126.
6. **Benigno M, Anastassopoulos KP, Mostaghimi A, Udall M, Daniel SR, Cappelleri JC, Chander P, Wahl PM, Lapthorn J, Kauffman L, Chen L (2020):** A large cross-sectional survey study of the prevalence of alopecia areata in the United States. Clinical, cosmetic and investigational dermatology. (13):259.
7. **Bhat YJ, Latif I, Malik R, Hassan I, Sheikh G, Lone KS, Majeed S, Sajad P (2017):** Vitamin D level in alopecia areata. Indian journal of dermatology. 62(4):407.
8. **Breitkopf T, Leung G, Yu M, Wang E, McElwee KJ (2013):** The basic science of hair biology: what are the causal mechanisms for the disordered hair follicle?. Dermatologic clinics. 31(1):1-9.
9. **Chelidze K & Lipner SR (2018):** Nail changes in alopecia areata: an update and review. International journal of dermatology, 7(57): 776-783.
10. **Corazza GR, Andreani ML, Venturo N, Bernardi M, Tosti A, Gasbarrini G (1995):** Celiac disease and alopecia areata: report of a new association. Gastroenterology. 109(4):1333-7.
11. **Dainichi T & Kabashima K (2017):** Alopecia areata: what's new in epidemiology, pathogenesis, diagnosis, and therapeutic options? Journal of dermatological science, 1(86), 3-12.
12. **Denham JM & Hill ID (2013):** Celiac disease and autoimmunity: review and controversies. Curr Allergy Asthma Rep, 4(13), 347-53.
13. **El Gayyar MA, Helmy MI, Abdelhafez A, Omran NA, Amer ER (2011):** Evaluation of thyroid hormone abnormalities and thyroid autoantibodies in chronic idiopathic urticaria and alopecia areata Egyptian patients. Asian J Dermatol. 3(1):1-2.
14. **Elli L, Bonura A, Garavaglia D, Rulli E, Floriani I, Tagliabue G, Contiero P, Bardella MT (2012):** Immunological comorbity in coeliac disease: associations, risk factors and clinical implications. Journal of clinical immunology. 32(5):984-90.
15. **Ertekin V, Tosun MS & Erdem T (2014):** Screening of celiac disease in children with alopecia areata. Indian J Dermatol, 3(59): 317.
16. **Fricke ACV & Miteva M (2015):** Epidemiology and burden of alopecia areata: a systematic review. Clinical, cosmetic and investigational dermatology, (8): 397.
17. **Gilhar A, Etzioni A & Paus R (2012):** Alopecia areata. New England Journal of Medicine. 366(16):1515-25.
18. **Hallaji Z, Akhyani M, Ehsani AH, Noormohammadpour P, Gholamali F, Bagheri M, Jahromi J (2011):** Prevalence of anti-gliadin antibody in patients with alopecia areata: a case-control study. Tehran University Medical Journal. 68(12).
19. **Hujoel IA, Reilly NR & Rubio-Tapia A (2019):** Celiac disease: clinical features and diagnosis. Gastroenterology Clinics, 1(48), 19-37.
20. **Jabbari A, Cerise JE, Chen JC, Mackay-Wiggan J, Duvic M, Price V, Hordinsky M, Norris D, Clynes R, Christiano AM (2016):** Molecular signatures define alopecia areata subtypes and transcriptional biomarkers. EBioMedicine. (7):240-7.

21. **Jabri B & Abadie V (2015):** IL-15 functions as a danger signal to regulate tissue-resident T cells and tissue destruction. Nat Rev Immunol. 15(12):771-83.

22. **Jang YH, Park KH, Kim SL, Lim HJ, Lee WJ, Lee SJ (2015):** Alopecia areata in the elderly: a 10-year retrospective study. Annals of dermatology. 27(4):411-6.

23. **Karnsakul W, Skitarelic K, Gillespie S, Arkachaisri T (2012):** Isolated positive anti-gliadin immunoglobin-A antibody in children with gastrointestinal symptoms. Turk J Gastroenterol. 23(5):485-9.

24. **Kim KH, Kwon SH, Sim WY, Lew BL (2020):** Characterization of a cohort of 294 patients with late-onset alopecia areata. Journal of the American Academy of Dermatology. 23.

25. **Kinori M, Kloepper JE & Paus R (2011):** Can the hair follicle become a model for studying selected aspects of human ocular immune privilege?. Investigative ophthalmology & visual science. 52(7):4447-58.

26. **Lebwohl B, Sanders D S & Green PH (2018):** Coeliac disease. The Lancet, 10115(391), 70-81.

27. **Lee NR, Kim BK, Yoon NY, Lee SY, Ahn SY, Lee WS (2014):** Differences in comorbidity profiles between early-onset and late-onset alopecia areata patients: a retrospective study of 871 Korean patients. Annals of dermatology. 26(6):722-6.

28. **Leonard MM, Sapone A, Catassi C, Fasano A (2017):** Celiac disease and nonceliac gluten sensitivity: a review. Jama. 318(7):647-56.

29. **Lundin M, Chawa S, Sachdev A, Bhanusali D, Seiffert-Sinha K, Sinha AA (2014):** Gender differences in alopecia areata. Journal of drugs in dermatology: JDD. 13(4):409-13.

30. **Lyakhovitsky A, Aronovich A, Gilboa S, Baum S, Barzilai A (2019):** Alopecia areata: a long-term follow-up study of 104 patients. Journal of the European Academy of Dermatology and Venereology. 33(8):1602-9.

31. **Lyakhovitsky A, Gilboa S, Eshkol A, Barzilai A, Baum S (2017):** Late-onset alopecia areata: a retrospective cohort study. Dermatology. 233(4):289-94.

32. **McElwee KJ, Gilhar A, Tobin DJ, Ramot Y, Sundberg JP, Nakamura M, Bertolini M, Inui S, Tokura Y, King LE, Duque-Estrada B (2013):** What causes alopecia areata? Section Editors: Ralf Paus, Manchester/Lübeck and Raymond Cho, San Francisco. Experimental dermatology. 22(9):609-26.

33. **Mirzoyev SA, Schrum AG, Davis MD, Torgerson RR (2014):** Lifetime incidence risk of Alopecia Areata estimated at 2.1 percent by Rochester Epidemiology Project, 1990–2009. The Journal of investigative dermatology. 134(4):1141.

34. **Mokhtari F, Panjehpour T, Naeini FF, Hosseini SM, Nilforoushzadeh MA, Matin M (2016):** The frequency distribution of celiac autoantibodies in alopecia areata. International journal of preventive medicine. 7.

35. **Olsen EA (2011):** Investigative guidelines for alopecia areata. Dermatol Ther. 24:311–319.

36. **Pratt CH, King LE, Messenger AG, Christiano AM, Sundberg JP (2017):** Alopecia areata. Nature reviews Disease primers. 3(1):1-7.

37. **Putterman E, Patel DP, Andrade G, Harfmann KL, Hogeling M, Cheng CE, Goh C, Rogers RS, Castelo-Soccio L (2019):** Severity of disease and quality of

life in parents of children with alopecia areata, totalis, and universalis: a prospective, cross-sectional study. Journal of the American Academy of Dermatology. 80(5):1389-94.

38. **Ragab MA, Hassan EM, El Niely DA, Mohamed MM (2020):** Serum level of interleukin-15 in active alopecia areata patients and its relation to age, sex, and disease severity. Advances in Dermatology and Allergology/Postępy Dermatologii i Alergologii. 37(6):904.
39. **Rodrigo L, Beteta-Gorriti V, Alvarez N, Gómez de Castro C, De Dios A, Palacios L, Santos-Juanes J (2018):** Cutaneous and mucosal manifestations associated with celiac disease. Nutrients. 10(7):800.
40. **Rubio-Tapia A, Hill ID, Kelly CP, Calderwood AH, Murray JA (2013):** American College of Gastroenterology clinical guideline: diagnosis and management of celiac disease. The American journal of gastroenterology. 108(5):656.
41. **Sapone A, Bai JC, Ciacci C, Dolinsek J, Green PH, Hadjivassiliou M, Kaukinen K, Rostami K, Sanders DS, Schumann M, Ullrich R (2012):** Spectrum of gluten-related disorders: consensus on new nomenclature and classification. BMC medicine. 10(1):1-2.
42. **Seirafi H, Ehsani A, Sadat Hosseini M, Samavati B, Gholamali F, Noormohammadpour P (2013):** Comparison of thyroid function tests in alopecia totalis and universalis with control group. Tehran University Medical Journal. 71(4).
43. **Tan E, Tay YK & Giam YC (2002):** A clinical study of childhood alopecia areata in Singapore. Pediatr Dermatol, 4(19): 298-301.
44. **Usatine RP, Smith MA, Mayeaux EJ, Chumley H (2019):** The color atlas and synopsis of family medicine. McGraw Hill Professional.
45. **Villasante AC & Miteva M (2015):** Epidemiology and burden of alopecia areata: a systematic review. Clin Cosmet Investig Dermatol, (8):397-403.
46. **Wu MC, Yang CC, Tsai RY, Chen WC (2013):** Late-onset ٩ areata: a retrospective study of 73 patients from Taiwan. Journal of the European Academy of Dermatology and Venereology. 27(4):468-72.
47. **You HR & Kim SJ (2017):** Factors Associated with Severity of Alopecia Areata. Annals of dermatology, 5(29), 565-570.

تقييم مستوى الأجسام المضادة لمضادات الغليادين فئة ألف وجيم في سيرم مرضي الثعلبة البقعية وعلاقتها بدرجة شدة المرض

***آلاء مكرم محمود عبد الوهاب، *حنـان محمد على درويـش،**

***هـدى كمال السبـــاعى، **ساره يونس عبد العزيز**

***قسم الأمراض الجلدية والتناسلية، كلية طب بنات، جامعة الأزهر**

****قسم الباثولوجيا الاكلينيكيه، كلية طب بنات، جامعة الأزهر**

E-mail: dr.alaa.makram01@gmail.com

خلفيـــة البحـــث: داء الثعلبـــة هـــو مـــرض مـــن أمـــراض المناعـــة الذاتيـــة تتوســـطه الخلايـــا اللمفاويـــة التائيـــة تلعـــب فيـــه المستضـــدات الذاتيـــة دورًا مهمًـــا فـــي تنشـــيط الخلايـــا التائيـــة وتـــرتبط بـــأمراض المناعـــة الذاتيـــة الأخـــرى. بصـــيلات الشـــعر هـــي موقـــع ذو امتيـــاز منـــاعي حيـــث لا يـــتم التعبيـــر عـــن جزيئـــات الصـــنف الأول والثـــاني مـــن معقـــد التوافـــق النســـيجي الرئيســـي (MHC). الـــداء البطنـــي هـــو اضـــطراب منـــاعي ذاتـــي يصـــيب الأشـــخاص المهيئـــين وراثيًـــا لتطـــوير رد فعـــل منـــاعي تجـــاه الغلـــوتين. الأمعـــاء الدقيقـــة هـــي الموقـــع الرئيســـي للـــداء البطنـــى، مـــن ناحيـــة أخـــرى، يمكـــن تمييـــزه عـــن طريـــق الاعـــراض المعويـــة واعـــراض خـــارج الأمعـــاء. يوصـــى فـــي البدايـــة بـــإجراء الاختبـــارات المصـــلية لتشـــخيص الـــداء البطنـــى. أحـــد الاختبـــارات الأكثـــر حساســـية وتحديـــدًا هـــو مضـــاد ترانســـجلوتاميناز ألـــف للأنســـجة. تشـــمل الاختبـــارات المصـــلية الأخـــرى مضادات الغليادين ألف وجيم.

الهـدف مـن البحـث: تقيـيم مسـتوى الأجسـام المضـادة لمضـادات الغليـادين فـي الـدم فئـة ألف وجيم في مرضي الثعلبة البقعية وعلاقتها بدرجة شدة المرض.

المرضـــي وطـــرق البحـــث: تضـــمنت دراســـة الحالـــة هـــذه 60 مشـــاركًا بمـــا فـــي ذلـــك 30 مريضًـــا يعـــانون مـــن داء الثعلبـــة و 30 فـــردًا ســـليمًا كعناصـــر تحكـــم. تـــم اختيـــار المرضـــى مـــن العيـــادة الخارجيـــة للأمـــراض الجلديـــة فـــي مستشـــفى الزهـــراء الجـــامعي خلال الفترة من نوفمبر 2020 إلى أبريل 2021.

نتـــائج البحـــث: مـــن بـــين 30 مريضًـــا يعـــانون مـــن داء الثعلبـــة المدرجـــة فـــي الدراســـة، كانـــت درجـــة شـــدة أداة الثعلبـــة (SALT) هـــي S5 فـــي 8 (26.7٪) مرضـــى، S4 فـــي 6.72) ٪ (، S3فـــي 3.31) ٪ (، S2فـــي 5 (16.7٪) و S1 فـــي 14 (46.7٪). كـــان مضـــاد الغليـــادين ألـــف إيجابيًـــا فقـــط فـــي 8 (26.7٪) مرضـــى S5 بينمـــا كـــان مضـــاد الغليـــادين جـــيم إيجابيًـــا فـــي جميـــع المرضـــى الثلاثـــين مـــع حـــالتين إيجـــابيتين فـــي مجموعـــة الـــتحكم (6.7٪). كـــان هنـــاك فـــرق ذو دلالـــة إحصـــائية بـــين مجموعـــة الـــتحكم والمرضـــى فيمـــا يتعلـــق بمســـتويات ألـــف وجـــيم، حيـــث تـــم العثـــور علـــى قـــيم أعلـــى فـــي المرضـــى مـــن المجموعة الضابطة.

الأســـتنتاج: كـــان هنـــاك ارتبـــاط ذو دلالـــة إحصـــائية عاليـــة بـــين الأجســـام المضـــادة للغليـــادين (فئـــة ألـــف و جـــيم)، و الثعلبـــة وشـــدتها، مـــع حساســـية وخصوصـــية 100٪ للفئـــه ألـــف مـــع نقطـــة قطـــع> 11.56 وأيضًـــا للفئـــه جـــيم بنقطـــة قطـــع> 41.8. يوصـــى بالبحـــث النشـــط عـــن الـــداء البطنـــى باســـتخدام اختبـــارات الفحـــص المصـــلي لتشـــخيص الحالات العديدة لمرضي الداء البطنى تحت الإكلينيكي في مرضى الثعلبة البقعية.

الكلمـــات الدالـــة: الأجســـام المضـــادة لمضـــادات الجلاديـــن، فئـــة ألـــف وفئـــة جـــيم، داء الثعلبة.