Aswan University Medical Journal, volume 4 / No.2/ September 2024 (80-86) Online ISSN: **2735-3117**

ORIGINAL ARTICL

Evaluation Level Of Serum Adiponectin In Patients With Alopecia Areata

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ABSTRACT

Keyword: Adiponectin, Alopecia Areata, SALT Score.

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Background: Alopecia areata (AA) is an autoimmune disease (AID) marked by transient hair loss wihout scaring. Approximately 2.3% of Egyptian population suffer from AA. Adiponectin is an adipokine secreted from adipocytes and plays a role in numerous physiological processes and cellular activities, such as lipid metabolism, energy management, immune response, inflammation, and insulin sensitivity. Additionally, it provides protective benefits to neurons and neural stem cells. **Objectives**: The study aimed to evaluate serum levels of Adiponectin in AA patients and to compare serum level of Adiponectin between AA cases and healthy controls. **Patients and methods**: This was a case-control study included 69 respondents; 39 cases (patients with AA)20 females(51.3%) ,and 19 males (48.7%) and 30 controls 18 females(60%) and 12males (40%). we assessed Adiponectin serum levels of the healthy controls and cases with AA by using Enzyme-linked immunosorbent assay (ELISA). All participants underwent clinical examination, history taking. **Results:** Serum Adiponectin levels in patients with AA were notably lower compared to those in the healthy control group. **Conclusion:** It was found that with one-ng/ml increase in the serum Adiponectin level there was 19% decrease the chance of having AA.

INTRODUCTION

Alopecia areata (AA) is an AID marked by transient non-scarring alopecia (1). Approximately 2.3% of Egyptian population suffer from AA. Compared to adults, children are more likely to have this condition, and its prevalence has been seen to increase over time. (2). The actual reason of AA is still unclear, but it's currently believed to arise from the disturbance of immune privilege in hair follicles caused by immune system activity. Genetic and environmental factors have an essential role in AA development. (3). The onset of AA is linked to the stimulation of innate and adaptive immune cells around the hair follicles, causing the interruption of normal hair follicle function. (4). Alopecia areata is mainly diagnosed through the meticulous clinical examination of the affected area. When there is doubt, trichoscopy can be a valuable non-invasive method to assist in the diagnosis, minimizing the requirement for more invasive approaches such as biopsies. (5). The commonest clinical feature of alopecia areata is the presence of at least one patch that is round or oval and totally hairless, resulting in a smooth and bald appearance (6). AA patients show unusual levels of adipokines, specifically adiponectin and resistin, in their blood. Adiponectin could serve as an indicator of the degree of hair loss in AA, indicating its severity. The compromised release of specific adipokines may

have a significant and multifaceted involvement in the development and progression of AA (7). Adiponectin is a circulating hormone produced by the adipocytes that is essential for maintaining homeostasis. It regulates glucose levels, insulin sensitivity, and lipid metabolism via its anti-inflammatory, anti-fibrotic, and antioxidant effects. Adiponectin mediates such metabolic processes through its 2 receptors, AdipoR1 and AdipoR2. Additionally, one of the hormones with the greatest plasma concentrations is adiponectin. (8)

AIM OF THE WORK: The study aimed to evaluate levels of serum Adiponectin in patients with AA , and compare serum level of Adiponectin between AA patients with control healthy group.

PATIENTS AND METHODS:

The study was a case-control design involving 69 subjects: 39 cases with AA (20 females, 51.3%, and 19 males, 48.7%) and 30 controls (18 females, 60%, and 12 males, 40%). All subjects were enrolled from the outpatient clinic of the Dermatology, Venereology, and Andrology Department at Aswan University Hospitals. The study was conducted from January 2022 to January 2023.All patients received an informed consent and approval for the research was obtained from the IRB of Faculty of Medicine, Aswan University, IRB number 594/1/22.

Inclusion criteria:

1. AA patients of both sexes.
2. There were no restrictions based on age, race, or occupation.

Exclusion criteria:

1. Pregnant and lactating women, as well as immunocompromised patients.
2. Patients suffering from coagulation disorders.
3. Patients who applied topical or intradermal therapies in the past 28 days, or who had received systemic therapy for AA in the past three months.

Methods:

Patients were subjected to:

a. Complete history taking: involved recording details such as age, occupation, previous treatments, the course and duration of the condition, and any systemic diseases.

b. General and dermatologic examination: to rule out any systemic or accompanying AIDs.

c. Assessment of AA severity by using SALT score.

d. Adiponectin serum level assessment :

Blood samples (5 cc) were collected from the antecubital vein into serum separator tubes. The samples were allowed to clot for 10-20 minutes at room temperature (22 Celsius degree) prior to being centrifuged for 20 minutes at 2000-3000 r.p.m. After that, the he serum was separated and stored at -30°C till analysis. Commercial ELISA kits were used for the analysis, specifically the human Adiponectin (ADP) ELISA kit from Bioassay Technology Laboratory (catalog number E1550HU). The standard curve for the kits is 0.2-60mg/L, with a sensitivity of 0.11mg/L.

Assay procedure:

- ✓ In room temperature, 50ul of the standard were added to the standard wells, and 40ul of the sample were added to the sample wells. Then, 10ul of Human APM1 antibody were added to the sample wells, followed by 50ul of streptavidin-HRP to both the sample and standard wells (excluding the blank well).
- ✓ The contents were mixed thoroughly, the plate was covered with a sealer, and it incubation was done sixty min at 37°C.

- ✓ Following removal of the sealer, the plate was washed five times by using wash buffer. After that, the plate was blotted into absorbent material.
- ✓ Fifty ul of substrate solution A was added to all wells, followed by 50ul of substrate solution B. The plate was after that covered with another sterilized sealer and incubated for ten min at 37°C in a dark room.
- ✓ Next, 50 ul of stop solution were added to all wells, causing an immediate change of the blue colour into a yellowish one. The OD of each well was measured by utilizing a microplate reader set to 450nm within ten min of adding the stop solution.

Statistical analysis:

The collected data were coded, and analysed using IBM-SPSS 21.0 (9).

Descriptive statistics were conducted by measurement of Means±SD, medians, ranges, and percentages. We used The Chi-square test to compare the differences of frequencies among the studied groups. Shapiro-Wilk test was utilized to test for data normality. Multivariate regression was measured to assess the significant predictors of ASB (OR -OR-, 95% CI- and p-value-). McNemar test was used to compare proportions within group on repeated measures. A significant p value was set at 0.05.

Sample size calculation

The required number of cases was determined by utilizing G*Power 3 software **(10)** , with a power of eighty percent and type I error of 5% (α=0.05 and β=80%) on two tailed test, the minimum needed sample was 69 subjects .This was split into two groups: **Group I** (AA patients, n=39) and **Group II** (healthy control group, n=30).

RESULTS

As shown in **table 1**,AA cases and healthy controls were matched for age (p=0.765), sex (p=0.470) and BMI (p=0.763).

Parameter	Case (n = 39)	Control (n = 30)	P-value
Age/years	19.74 ± 2.9	20.57 ± 1.9	= 0.765*
Sex			
• **Female**	20 (51.3%)	18 (60%)	= 0.470**
• **Male**	19 (48.7%)	12 (40%)	
BMI	20.84 ± 5.4	20.46 ± 4.7	= 0.763*

***Independent Sample t-test, **Chi-square test**

The median Adiponectin level was significantly (p<0.001) lower in cases (4.2 (2.7-23) ng/ml) compared with control (7.8 (3.8-23) ng/ml) as shown in **Table 2 and Fig. 1**.

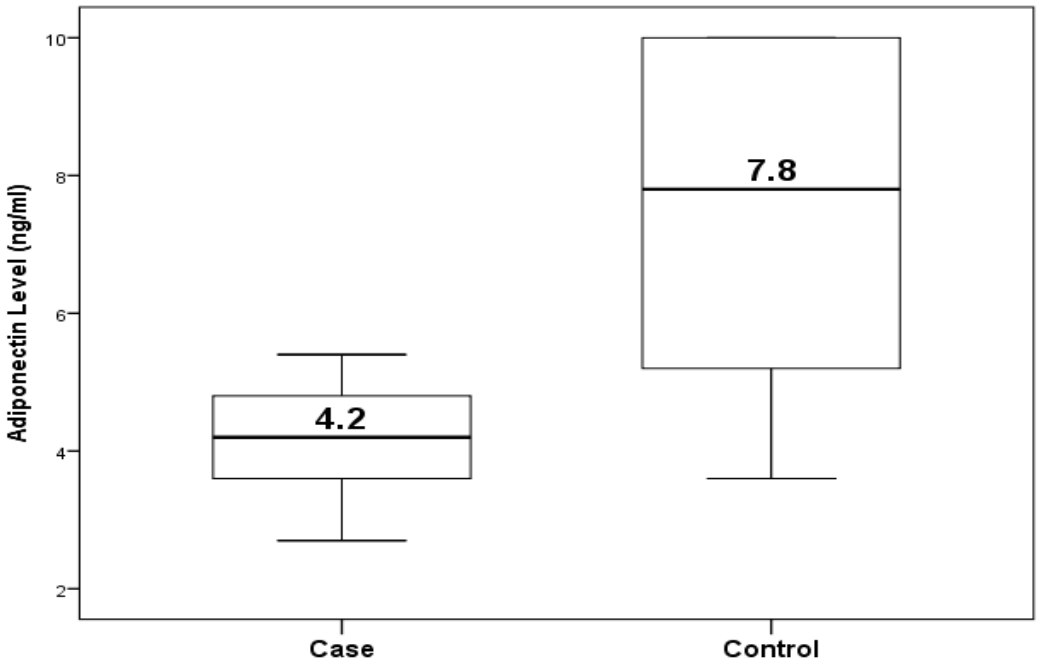
Table 2 : The differences in the Adiponectin Level between Groups

Parameter	Cases (n = 39)	Control (n = 30)	P-value
Adiponectin Level (ng/ml)			
• **Mean ± SD**	5.21 ± 3.8	7.92 ± 7.2	**< 0.001***

• **Median (Range)**	4.2 (2.7 - 23)	7.8 (3.8 - 23)

***Kruskal Wallis test, **Post-hoc test with Bonferroni corrections**

Fig. 1: Difference in the Median Adiponectin Level among the studied Groups



It was found that with one-ng/ml increase in the serum Adiponectin level there was 19% (OR=0.81, 95% CI; 0.706 – 0.942) decrease the chance of having AA and this was statistically insignificant (p = 0.006) **table 3**

Table 3: Independent Effect of Adiponectin Level on AA Disease:

	OR (95% CI) *	P-value
• **Age/years**	0.988 (0.909 – 1.074)	= 0.780
• **Sex (Male)**	1.061 (0.358 – 3.148)	= 0.915
• **BMI**	1.046 (0.878 – 1.274)	= 0.614
• **Adiponectin (ng/ml)**	0.813 (0.706 – 0.942)	**= 0.006**

OR=Odds Ratio; CI, Confidence Interval

DISCUSSION

AA is an AID that interferes with the immune protection of hair follicles.**(11).** The prevalence of metabolic abnormalities in individuals with AA, along with the potential targeting of melanocytes by activated cells in the condition, indicates a potential involvement of

adipokines in AA development. This recommends that adipokines may have a main function with regard to AA pathogenesis **(7)**.

Adiponectin is a fairly common protein found in human serum. On the other hand, its concentrations decline in different pathological disorders including, metabolic syndrome, insulin resistance, cardiac disorders, and obesity **(8)**

The current study revealed that the median level of Adiponectin was significantly ($p<0.001$) lower in cases (4.2 (2.7-23) ng/ml) compared to control (7.8 (3.8-23) ng/ml)

On the same way, **Stochma et al.** noticed that there was negative association between SALT score and Adiponectin value in cases with AA **(7).**

In contrast, **Serarslan et al.** noticed that Adiponectin level was higher among patients with scalp AA compared to the controls, but there was no difference in its level between beard and eye brow alopecia patients and control group **(12)**.

There is a suggestion that Adiponectin could potentially serve as a regulated and selective modulator of the inflammatory response **(14).**

Adiponectin plays significant roles in regulating the immunity. In contrast, there is ongoing debate regarding whether it causes its action by pro-inflammatory action or anti-inflammatory effects **(13)**.

Adiponectin enhances the transformation of monocytes into M2 macrophages and inhibits their transformation into M1 macrophages, which display anti-inflammatory and pro-inflammatory properties, respectively **(8)**.

M1 macrophages are characterized by their ability to trigger pro-inflammatory factors and contribute to the development of insulin resistance. Conversely, M2 macrophages inhibit inflammatory responses and promote oxidative metabolism. Adiponectin acts by impeding the activation of M1 macrophages while facilitating the activation of M2 macrophages **(14).**

In line with our results, adiponectin has been observed to promote the growth of scalp hair in ex-vivo research **(15)**.

Our study goes along with **Hagino T et al.** Who reported that the reduction of Adiponectin, an anti-inflammatory adipokine, could potentially play a role in promoting autoimmune inflammation **(16).**

This result also is identical with the Egyptian study done by **Farag et al**. Who found that Adiponectin serum values were markedly lower in AA cases compared to the controls ($p=0.001$)**(17).**

CONCLUSION

We concluded that following adjusting for age, gender, and BMI, it was found that with one-ng/ml increase in the serum Adiponectin level there was 19% decrease the chance of having AA.

LIMITATIONS

The relatively small sample size and brief study duration point to considerable disparities between our findings and those of the other investigations.

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