Article

Study the Levels of Adrenaline and Prolactin as a Stress Monitoring Biomarkers in the Patients with Alopecia Areata in Babylon Governorate

Rasool Mahmoud Hashem [1]* ,Abdulsamie Hassan Alta'ee [2] and Wessam Ali Ameen [2]

1 General Directorate of Health in Najaf Al-Ashraf
2 College of Medicine, University of Babylon, Babylon, Hilla P Box 473.
* Corresponding author email: saidrasol79@gmail.com

Abstract

Background: Men and women are equally affected by the common, non-scarring, inflammatory kind of hair loss known as alopecia areata. It has a 2% cumulative lifetime incidence and affects 0.1% to 0.2% of the general population in the US. Moreover, a major cause of hair loss is stress. As a result, the adrenal gland releases catecholamines and glucocorticoids, which have an impact on the vasculature to raise blood pressure, the metabolism to supply quick energy, and the immune system by inhibiting extended activation.

Objective: Investigating the possible involvement of stress in the pathophysiology of alopecia areata is the goal of this study. Furthermore, to clarify the possible application of a stress monitoring biomarker as an alopecia areata diagnostic indication.

Materials and methods: Sixty male patients with alopecia areata and sixty male controls who seemed to be in excellent health participated in a case-control study. Furthermore, the alopecia areata patients were divided into two age groups: adults and non-adults. Adrenaline and prolactin levels were measured using blood samples. Along with other characteristics, age and BMI were assessed. By dividing weight in kilograms by height squared, the BMI was computed. Studies utilizing statistical methods, such as Pearson correlation and ROC-curve analysis, were conducted to assess the diagnostic precision of prolactin and adrenaline.

Results: The results of this study indicated that the patient group's adult adrenalin and prolactin concentrations rose considerably ($p<0.05$), but the child's serum prolactin levels did not significantly change between the two groups (p-value >0.05).

Conclusions: This study suggests that prolactin and adrenaline may play important roles in the pathogenesis of alopecia areata (AA).

Keywords: alopecia areata, Adrenaline, Prolactin, ROC-Curve, Iraq.

Introduction

Alopecia areata (AA) is an autoimmune disorder that results in temporary hair loss without scarring, although the hair follicle itself remains intact. Hair loss can occur in all body regions where hair grows and can manifest as complete, partial, or well-defined patches [1]. One to two percent of the general population may ultimately become affected by the disease. Skin biopsies from alopecia areata patients show that lymphocytes are present in and around the bulb, or bottom section, of the hair follicle during the anagen (hair growth) phase. It is believed that a disruption in the hair follicle's immunological privilege is a significant contributing cause to AA [2]. It is believed that AA affects people of all ages; among Saudi patients, its total prevalence is 2.3% [3]. Psychological stress activates the hypothalamic-pituitary-adrenal (HPA) and brain-hair follicle (BHA) axis. The hypothalamic hormones corticotrophin-releasing hormone (CRH) and adrenocorticotrophic hormone (ACTH) work together to control cortisol release through the hypothalamus-pituitary-adrenal axis [4].

Because of its many uses as a hormone and neurotransmitter, adrenaline, sometimes referred to as epinephrine, can also be used as medicine. It is typically generated by a limited number of neurons in the medulla oblongata and the adrenal gland as part of the sympathetic nervous system's fight-or-flight response [5].

The sympathetic response of the organism is ultimately mediated by the adrenergic receptors' activation of ion channels via either the phosphoinositol or cyclic adenosine monophosphate (cAMP) second messenger systems.The first step in the synthesis is the conversion of phenylalanine to L-tyrosine, which is then changed by tyrosine hydroxylase into L-dopa. Afterwards, L-aromatic amino acid decarboxylase converts L-dopa to dopamine [6]. Due to its effects on the Sinoatrial node (SA), blood sugar level, muscular blood flow, pupil dilation response, and cardiac output, adrenaline is a vital component of the fight-or-flight response. Through its binding to alpha and beta receptors, these effects are carried out. Additionally, it affects metabolic pathways in a variety of ways. For example, it can activate protein kinase A (PKA) and increase hormone-sensitive lipase (HSL), which in turn promotes lipolysis [7].

Prolactin (PRL) is a polypeptide hormone, also known as luteotropin, that is primarily generated in the autocrine/paracrine and endocrine systems by the anterior pituitary gland's lactotroph cells [8]. Human skin and hair follicles both generate prolactin, which is now recognized to play a significant role in human cutaneous biology. This function includes controlling epithelial stem cell activity and keratin expression through hair growth modulation. In particular, prolactin enhances the proliferation of epidermal keratinocytes in cell culture, upregulates keratin expression in adult human epithelial stem cells ex vivo, and increases hair development in human hair follicle organ culture [9].

This study aims to clarify the possible application of a stress monitoring biomarker as a diagnostic criterion for AA and explore the role that stress may have in the pathogenesis of AA.

Materials and Methods

Out of the 120 participants in this case-control study, 60 had alopecia areata and the other 60 appeared to be in good condition. Every sample was taken between March 2024 and September 2023. Patients who had reported visits to the dermatology clinics at the Imam Al-Sadiq Teaching Hospital, the Marjan Teaching Hospital, and the Hilla General Teaching Hospital provided samples.

The patients ranged in age from under eighteen to over eighteen. A lot of data, including the patient's history, height, weight, age, duration, and severity, was recorded. Dermatologist physicians used selection and exclusion criteria to diagnose patients and categorize patient groups, such as smokers, those who had just taken medicine, and those with chronic illnesses including cancer, diabetes, and hypertension.

Each individual had a vein in their body sampled. After gently pumping the blood into a gel tube and letting it clot for ten to fifteen minutes at room temperature, the sample was centrifuged for ten minutes at 3000 x g. Following serum extraction and transfer to Eppendorf tubes, the concentrations of prolactin and adrenaline were measured.

Determination of human Serum (Adrenaline and Prolactin) Concentration by ELISA

The manufacturer's instructions (Bioassay Technology Laboratory, respectively) were followed in order to assess the serum levels of adrenaline and prolactin using commercially available enzyme-linked immunosorbent assay (ELISA) kits.

Ethics approval

All research participants were informed and given the opportunity to give verbal permission prior to sample collection. The study protocol, subject details, and consent form were reviewed and authorized by a local college and hospital ethics committee under the document number [IRB: 1291, 16/8/2023].

Statistical analysis:

The mean difference between the two groups (p-value < 0.05), which is deemed significant, was determined statistically using the t-test in the current study, which compares the patient and control groups. The correlation between the factors under investigation was also ascertained using Pearson correlation. A ROC curve analysis was performed using the IBM Statistical Package for the Social Sciences (SPSS V. 26) for prolactin and adrenaline levels.

Results

a. The demographic characteristics of the study participants

The present comparative study of the patient and control groups used the t-test to statistically compute the mean difference between the control and alopecia areata groups as well as the association between the various patient parameters. Two control groups of thirty each comprised the remaining sixty adult and sixty kid research groups.

1. Age

The data showed no statistically significant variation ($p > 0.05$). The age distribution and sickness rate are shown in Table 1. The comparison is made between the mean of adult and pediatric patients (27.30± 8.49. 12.17 ± 2.77) and healthy controls (31.27 ± 9.14. 11.57 ± 3.39), respectively.

Table 1: Age means of the study groups

Study variable	Groups	N	Mean ± SD	p-value
Age adult	**Patient**	30	27.30 ± 8.94	0.095
	Control	30	31.27± 9.14	
Age children	**Patient**	30	12.17 ± 2.77	0.456
	Control	30	11.57 ± 3.39	

2.BMI

The results did not differ statistically significantly ($p > 0.05$). The BMI dispersion and illness rate are shown in Table 2. A comparison is made between the means of adult and pediatric patients (22.40 ± 2.31. 19.767 ± 1.91) and healthy controls (23.03 ± 1.90. 20.662 ± 1.81), respectively.

Table2: BMI means of the study groups

Study variable	Groups	N	Mean ± SD	p-value
BMI adult	**Patient**	30	22.40 ± 2.31	0.252
	Control	30	23.03 ± 1.90	
BMI children	**Patient**	30	19.767 ± 1.91	0.068
	Control	30	20.662 ± 1.81	

b. Biochemical parameters

Table 3 displays a substantial ($p<0.05$) rise in the patient group's concentrations of adrenaline and prolactin in both adult and pediatric subjects when compared to the control group.

Table 3: Comparison of Adrenaline and Prolactin levels between study participants

Variable	N	Patients (Mean ± SD)	Control (Mean ± SD)	P-value
Adrenaline (ng/L)in adult	30	0.577 ± 0.280	0.1812 ± 0.033	0.000
Adrenaline (ng/L) in children		0.252 ± 0.167	0.107 ± 0.038	0.001
Prolactin (µIU/mL) in adult	30	0.496 ± 0.242	0.217 ± 0.086	0.000
Prolactin (µIU/mL) in children		0.487 ± 0.241	0.432 ± 0.212	0.000

c- Correlations between study parameters

Table 4: Pearson correlation among the study variables in the adult patients group

Study variables		Adrenalin	Prolactin	Age	BMI
Adrenalin	R	----	-.232	.324	-.144
	p-value	----	.217	.081	.448
Prolactin	R	-.232	----	-.339	.171
	p-value	.217	----	.067	.366
Age	R	.324	-.339	----	.291
	p-value	.081	.067	----	.119
BMI	R	-.144	.171	.291	----
	p-value	.448	.366	.119	----

Table 5: Pearson correlation among the study variables in the children patients group

Study variables		Adrenalin	Prolactin	Age	BMI
Adrenalin	R	----1	.293	.257	-.008
	p-value	----	.116	.171	.967
Prolactin	R	.293	----	-.086	-.161
	p-value	.116	----	.651	.394
Age	R	.257	-.086	----	.029
	p-value	.171	.651	----	.881
BMI	R	-.008	-.161	.029	----
	p-value	.967	.394	.881	----

Table 6: Area under the curve for adrenalin between the adult patient and control group

Area Under the Curve						
Test Result Variable: **adrenalin**						
AUC	**Specificity**	**Sensitivity**	**SE**	**p-value**	**95% Confidence Interval**	
					Lower Bound	**Upper Bound**
0.997	100%	97%	0.004	**0.000**	0.99	1
SE-standard error, AUC-area under the curve						

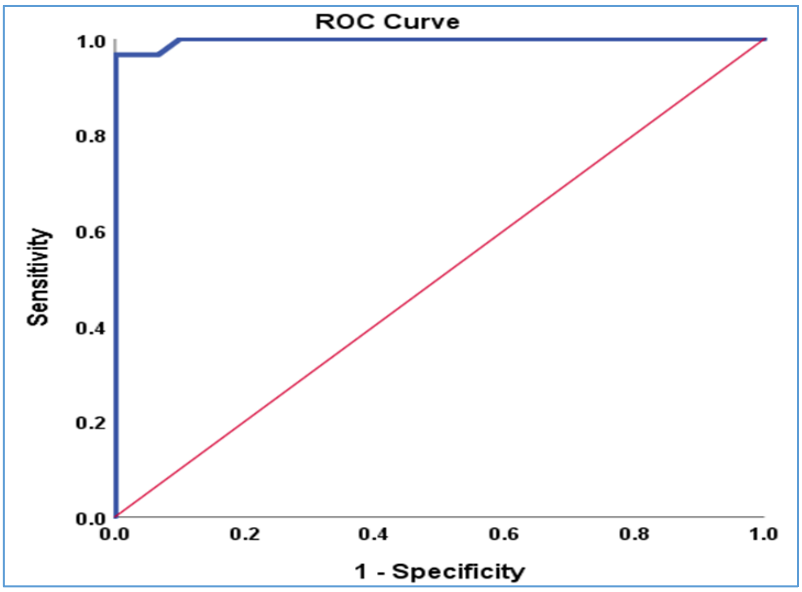

Figure 1: ROC curve analysis for adrenalin between the adult patients and control group

Table 7 : Area under the curve for adrenalin between the children patient and control group

Area Under the Curve						
Test Result Variable: adrenalin						
					95% Confidence Interval	
AUC	**Specificity**	**Sensitivity**	**SE**	**p-value**	**Lower Bound**	**Upper Bound**
.917	86%	86%	.036	**.000**	.847	0.988
SE-standard error, AUC-area under the curve						

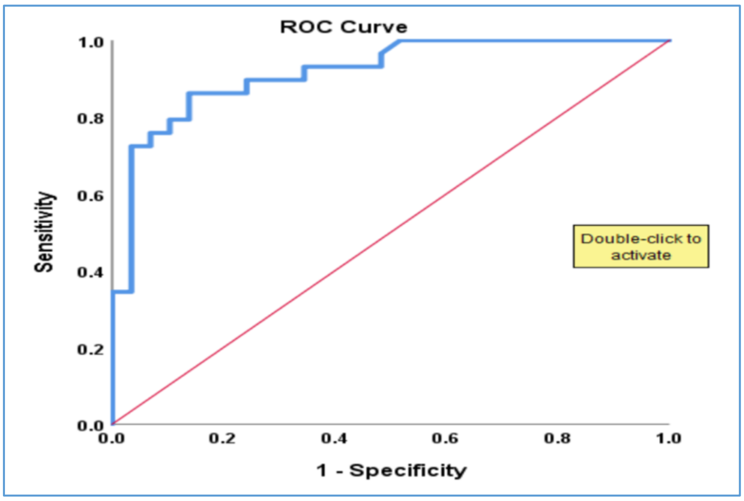

Figure 2: ROC curve analysis for adrenalin between the children patients and control group

Table 8: Area under the curve for prolactin between the adult patient and control group

Area Under the Curve						
Test Result Variable: **prolactin**						
					95% Confidence Interval	
AUC	Specificity	Sensitivity	SE	p-value	Lower Bound	Upper Bound
0.896	77%	90%	0.04	**0.000**	0.819	0.974
SE-standard error, AUC-area under the curve						

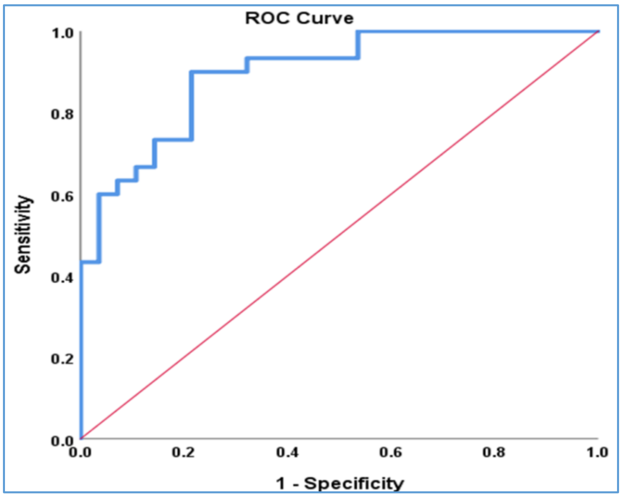

Figure 3: ROC curve analysis for prolactin between the adult patients and control group

Discussion

Table 3 illustrates that the mean levels of adrenalin in the adult patient and control groups differed significantly ($p<0.001$). In comparison to the control group, the adult patient group had greater levels of adrenalin, according to the results.

Table 3 indicates that there was a significant difference in the mean levels of adrenalin between the control group and the pediatric patients in the current study. The patients had higher levels than the control group (0.252 ± 0.167 vs. 0.107 ± 0.038, p-value <0.05).Raised levels of adrenaline in the adult and pediatric patient groups are not surprising, since it is well recognized that stress conditions play a significant role in the development of alopecia areata. Adrenaline is one of the stress hormones.[10] While several studies have shown the importance of stress in activating CRH in autoimmune cutaneous illnesses, including alopecia areata, which in turn results in adrenaline release, no other research has measured the levels of adrenaline in individuals with this condition [11].

Table 3 presents the study's findings, which indicate that the adult sick group had higher prolactin levels than the adult control group ($p<0.05$).This outcome is consistent with research on adult patients with alopecia undertaken by El-Farargya et al. 2020 and Elsherif et al. 2015 [12], [13]. The stress reaction that develops in AA patients may be the cause of the elevated prolactin levels. Furthermore, prolactin stimulates T and B cells, natural killer cells, neutrophils, macrophages, CD34+ hematopoietic cells, and antigen-presenting dendritic cells, all of which have a function in the immune system [14].

Furthermore, prolactin increases the amount of TUNEL + cells and decreases the number of Ki-67-positive cells in HF keratinocytes, which contributes significantly to their proliferation and apoptosis [15]. As a result, prolactin may be involved in the pathophysiology of AA. Table 3 indicates that there was no significant difference in blood prolactin levels between the children patients and control group (p-value >0.05). This finding may be explained by the age group of the patients and the potential hormonal changes associated with their age. As indicated in table 4,5, the current study's results indicated that there was no link (p-value >0.05) between age, BMI, prolactin, or adrenalin in the groups of adult and pediatric patients. Additionally, table 4,5 indicates that there is no association (p-value >0.05) between age, BMI, prolactin, or adrenalin in the adult patient group.

An evaluation of the adult patient group's adrenalin specificity and sensitivity at a cut-off value of 0.29 ng/l, with an AUC of 0.997, was conducted using the ROC curve analysis. For adrenaline, the sensitivity was 97% and the specificity was 100%. Table 6, Figure 1. When employed as a diagnostic marker for adult individuals with alopecia areata, the adrenalin AUC value is thought to be quite good.The ROC curve study also showed that the children's patient group's specificity and sensitivity to adrenalin was 0.917 AUC at the cut-off point of 0.141 ng/l when compared to the control group. Adrenaline exhibited 86% specificity and 86% sensitivity, in that order. Figure 2 in Table 7. Adrenaline's area under the curve serves as a very good diagnostic tool for pediatric AA patients.

The ROC curve analysis showed that the adult patient group's prolactin specificity and sensitivity were higher than those of the control group at the cut-off point of 0.263 ng/l, with an area under the curve of 0.896. For prolactin, the corresponding values were 90% and 77% for specificity and sensitivity. Table 8, Figure 3. According to this finding, the prolactin AUC value provides a good discriminant value for usage as a marker in adult AA patients.

Conclusion

The current study discovered that AA patients had higher levels of stress markers in adult participants. Conversely, there was no difference in prolactin and adrenaline levels between the adult and pediatric patient groups. Prolactin and adrenaline can be useful stress markers in AA patients.

References

[1] Sarah Isam Al-Rubaye, Abdulsamie Hassan Alta'ee, Zena Saeed Al-Fadhily, The Relationship between −330 Interleukin- 2 Gene Polymorphism and Its Plasma Levels in Patients with Alopecia Areata, Medical Journal of Babylon, 2019, 16, 292-295.

[2] Sarah Isam Al-Rubaye, Abdulsamie Hassan Alta'ee, Zena Saeed Al-Fadhily, The Association between (CT60) Cytotoxic T-lymphocyte Antigen-4 Gene Polymorphism with its Plasma Levels in Alpecia Areata Patients, Indian Journal of Public Health Research & Development, 2019, 10,10, 3567-3570.

[3] A. A. Alshahrani, R. Al-Tuwaijri, Z. A. Abuoliat, M. Alyabsi, M. I. Aljasser, and R. Alkhodair, "Prevalence and Clinical Characteristics of Alopecia Areata at a Tertiary Care Center in Saudi Arabia," Dermatol. Res. Pract., vol. 2020, 2020.

[4] Y. Katsu and M. E. Baker, "Cortisol," Handb. Horm. Comp. Endocrinol. Basic Clin. Res., pp. 947–949, Jan. 2021, doi: 10.1016/B978-0-12-820649-2.00261-8.

[5] S. Capellino, M. Claus, and C. Watzl, "Regulation of natural killer cell activity by glucocorticoids, serotonin, dopamine, and epinephrine," Cell. Mol. Immunol. 2020 177, vol. 17, no. 7, pp. 705–711, Jun. 2020, doi: 10.1038/s41423-020-0477-9.

[6] "PPT - PHARMACOLOGY OF SYMPATHETIC NERVOUS SYSTEM PowerPoint Presentation - ID:4101590." Accessed: Feb. 22, 2024. [Online]. Available: https://www.slideserve.com/helene/pharmacology-of-sympathetic-nervous-system

[7] B. J. G. Richard I.G Holt, Clive S. Cockram, Allan Flyvbjerg, Textbook of Diabetes, 4th Edition. A John Wiley & Sons, Ltd., Publication, 2010.

[8] C. L. Brooks, "Molecular mechanisms of prolactin and its receptor," Endocr. Rev., vol. 33, no. 4, pp. 504–525, 2012, doi: 10.1210/er.2011-1040.

[9] E. A. Langan, K. Foitzik-Lau, V. Goffin, Y. Ramot, and R. Paus, "Prolactin: An emerging force along the cutaneous-endocrine axis," Trends Endocrinol. Metab., vol. 21, no. 9, pp. 569–577, 2010, doi: 10.1016/j.tem.2010.06.001.

[10] D. Ahn, H. Kim, B. Lee, and D. H. Hahm, "Psychological Stress-Induced Pathogenesis of Alopecia Areata: Autoimmune and Apoptotic Pathways," Int. J. Mol. Sci. 2023, Vol. 24, Page 11711, vol. 24, no. 14, p. 11711, Jul. 2023, doi: 10.3390/IJMS241411711.

[11] X. Zhang, M. Yu, W. Yu, J. Weinberg, J. Shapiro, and K. J. McElwee, "Development of Alopecia Areata Is Associated with Higher Central and Peripheral

Hypothalamic–Pituitary–Adrenal Tone in the Skin Graft Induced C3H/HeJ Mouse Model," J. Invest. Dermatol., vol. 129, no. 6, pp. 1527–1538, Jun. 2009, doi: 10.1038/JID.2008.371.

[12] S. M. El- Farargya and H. N. A. H. , Naglaa M. Ghanayemb, "Clinical significance of serum prolactin levels in patients with alopecia areata," Menoufia Med. J., vol. 33, pp. 1011–1015, 2020, doi: 10.4103/mmj.mmj.

[13] N. A. Elsherif, A. I. El-Sherif, and S. A. El-Dibany, "Serum prolactin levels in dermatological diseases: A case–control study," J. Dermatology Dermatologic Surg., vol. 19, no. 2, pp. 104–107, 2015, doi: 10.1016/j.jdds.2015.03.002.

[14] S. Levine and O. Muneyyirci-Delale, "Stress-induced hyperprolactinemia: Pathophysiology and clinical approach," Obstet. Gynecol. Int., vol. 2018, 2018, doi: 10.1155/2018/9253083.

[15] K. Foitzik, K. Krause, F. Conrad, M. Nakamura, W. Funk, and R. Paus, "Human scalp hair follicles are both a target and a source of prolactin, which serves as an autocrine and/or paracrine promoter of apoptosis-driven hair follicle regression," Am. J. Pathol., vol. 168, no. 3, pp. 748–756, 2006, doi: 10.2353/ajpath.2006.050468.