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ORIGINAL ARTICLE

Claudin-3 is a novel intestinal integrity marker in patients with alopecia areata: Correlation with the disease severity

Dina Rafik MBBCh[1] | Ihab Younis MD[1] | Rana Atef MD[2] | Hanaa Eid MD[1]

[1]Dermatology Department, Faculty of Medicine, Benha University, Benha, Egypt

[2]Clinical Pathology and Chemistry Department, Faculty of Medicine, Benha University, Benha, Egypt

Correspondence
Hanaa Eid Abd Elaal, Department of Dermatology, Venereology & Andrology, Faculty of Medicine, Benha University, Benha, Egypt.
Email: hanaaeid90@gmail.com

Abstract

Background: The development of alopecia areata is suggested to be influenced by intestinal permeability and gut dysbiosis. Claudin-3, an essential component of tight junctions which may act as an indicator of intestinal barrier integrity.

Aims: The study's objective was to evaluate the plasma concentration level of Claudin-3 in alopecia areata patients and its relationship to the severity of the condition.

Patients and Methods: In this case–control study, 50 alopecia areata patients and 30 healthy age and sex controls were involved. An enzyme-linked immunosorbent assay was used to determine the concentration of claudin-3 in the blood.

Results: Patients with alopecia areata had significantly higher plasma claudin-3 concentrations than healthy controls [median (interquartile range), 7.73 ng/ml (4.49–33.7) vs. 6.14 ng/ml (4.45–15.6), $p < 0.005$]. Positive relations were found between claudin-3 and SALT score ($r = 0.675$ & p-value < 0.001).

Conclusions: Claudin-3, a gut permeability biomarker, is elevated in alopecia areata and correlates with disease severity.

KEYWORDS
alopecia areata, claudin 3, intestinal barrier

1 | INTRODUCTION

Non-scarring hair loss is a frequent immune-mediated disorder known as alopecia areata (AA). Prevalence of AA is between 1.7% and 2.1%, with a higher frequency in patients under the age of 40 (21–40), although there is no clear differentiation in incidence between men and women.[1]

Alopecia has various clinical subgroups, including Patch alopecia areata (PA), which causes hair loss in one or more patches, alopecia totalis (AT), that leads to hair loss throughout most of the scalp, and alopecia universalis (AU), that causes the majority of hair to fall out, including eyebrows and body hair.[2]

Experimental and clinical data suggest that alopecia areata is an example of an immune reaction on hair follicles that causes inflammation.[3] The generation and multiplication of autoreactive T cells and antibodies are the results of the immunological tolerance failure known as autoimmunity. However, the causes of such autoimmunity in AA are yet unknown.[4] There have been few researches done to study the function of intestinal microbiota in the pathophysiology of AA. Gut microbiota is suggested to have an important effect on immunoregulation under autoimmune situations like AA.[5] One case study mentioned hair development in two AA patients following microbiome transplantation that may indicate the role of intestinal microbiota in the pathophysiology of the disease.[6]

Physiological colonization of the gastrointestinal tract encourages the formation of barrier function, but dysbiosis impedes this process and increases gut permeability.[7] Immune activation comes from the transfer of pathogens, bacterial toxins, and metabolites into the peripheral circulation.[8] Tight junctions connect the integrated framework of epithelial cells that make up the gastrointestinal epithelial barrier.[9]

Claudins are transmembrane proteins that interact with the actin cytoskeleton to create tight junctions. Loss of intestinal barrier function results from the knockout of particular claudins. A great number of studies indicated that claudin-3 acts as a barrier forming tight junctional protein. Blood levels of Claudin-3 may serve as a measure for gut permeability.[10]

The purpose of this study was to evaluate Claudin-3 plasma concentration levels in alopecia areata patients and its correlation with the disease severity.

2 | MATERIALS AND METHODS

2.1 | Patients

The study included 50 alopecia areata patients (35 males and 15 women), who were sent to the dermatology outpatient clinic. Patients with localized and diffuse types of alopecia areata together with patients with newly developed lesions were included in the study. Patients who met any of the following conditions were excluded: any participant presenting with any other dermatological conditions or any psychiatric disorder, history of any systemic diseases, for example, liver diseases, diabetes mellitus, hyperlipidemia or hypertension, previous history of severe gastroenteritis or consumption of probiotics, prebiotics, or antibiotics or dietary limitations throughout the previous 3 months, previous six-month history of gastrointestinal surgery, long-term digestive illness (food allergies, irritable bowel syndrome, inflammatory bowel illness, celiac disease), pregnancy, and lactation.

All participants were subjected to a full medical history taking, a thorough general examination to exclude autoimmune diseases and local examination for Sites, number, morphology, and configuration of AA lesions. Severity of AA lesions was assessed using SALT score, in which the scalp is divided into the following 4 areas: Vertex: 40%, Right profile of scalp: 18%, Left profile of scalp: 18%, and Posterior aspect of scalp: 24% of scalp surface area. Percentage of hair loss in any of these areas is the percentage hair loss multiplied by percent surface area of the scalp in that area. SALT score is the sum of percentage of hair loss in all the abovementioned areas.

The control group included 30 participants, 19 males and 11 females, who were age and gender matched. The same exclusion criteria were applied to subjects in the control group. The study was accepted by the local medical ethical committee. Prior to enrollment and participation in the study, informed written consents were collected.

2.2 | Claudin 3

Each participant in the trial had five milliliters of venous blood drawn under strict aseptic conditions and placed in anticoagulant free test tubes. Samples were centrifuged after coagulation (at 1500 g for 15 min). The separated serum was aliquoted and kept at −20°C for the Claudin-3 assay. Human Claudin-3 immunoassay using a double-antibody sandwich ELISA kit was used to assess serum Claudin-3 levels (Sunredbio). Every measurement was done twice, and mean values were utilized to continue the analysis.

2.3 | Statistical analysis

The Kolmogorov–Smirnov test (for cases), the Shapiro–Wilk test (for controls), and direct data visualization methods were used to check the normality of quantitative data (for both). Means, deviations from the mean, or ranges were employed to summarize numerical data. Numbers and percentages were employed represent a categorical set of data. Depending on whether the quantitative data were regularly distributed or not, Mann–Whitney U- or independent t-tests were performed to compare the data between study groups. Chi-square test was employed to compare categorical data. ROC analysis was done for using claudin in diagnosing alopecia. Correlations were done using Spearman's correlation. Mann–Whitney U-test was employed to evaluate Claudin in accordance with several criteria. The prediction of alopecia was done using multivariate logistic regression analysis. There were two sides to each statistical test. p values ≤ 0.05 were considered to be significant.

3 | RESULTS

3.1 | Clinical features

Fifty alopecia areata patients and 30 control subjects participated in the study. Table 1 lists the key demographic, clinical, and biochemical information about the research cohort. Regarding age and sex, there were no statistically significant differences between patients and controls according to the study's design.

TABLE 1 General characteristics in both groups.

		Patients ($n = 50$)	Controls ($n = 30$)	p-Value
Age (Years)	Mean ± SD	27 ± 11	25 ± 9	0.479
Sex	Males n (%)	35 (70.0)	19 (63.3)	0.538
	Females n (%)	15 (30.0)	11 (36.7)	
Smoking	n (%)	19 (38.0)	8 (26.7)	0.299

Note: Independent t-test was used for age. Chi-square test was used for sex.

3.2 | Claudin-3

In comparison with healthy controls, patients with alopecia areata had significantly higher plasma concentrations of claudin-3. [7.73 ng/ml (4.49–33.7) vs. 6.14 ng/ml (4.45–15.6); $p < 0.005$].

ROC analysis was performed for serum claudin in diagnosing alopecia areata. It had a statistically significant AUC of 0.687 and a 95% confidence interval of 0.562–0.812 (p-value 0.001). The best cutoff point was >6.37, at which sensitivity and specificity were 82% and 56.7%, respectively (Figure 1).

According to the severity of alopecia tool score (SALT), we divided patients into four groups for subsequent analysis: mild disease (S1) (SALT ≤20; $n = 30$), moderate (S2) (SALT 21%–49%; $n = 13$), and severe disease (S3 & S4) (SALT ≥50% n = 7). Patients with mild, moderate, and severe alopecia had higher levels of plasma claudin compared to the control group (Figure 2).

3.3 | Interrelations

Claudin showed significant positive correlations with SALT score ($r = 0.675$ & p-value < 0.001). The median Claudin-3 level was significantly higher in those with sudden onset (7.76 ng/ml) than those with gradual onset (6.76 ng/ml); p-value was 0.034. Also, it was significantly higher in those with multiple lesions (9.48) than those with single lesions (7.07); p-value was < 0.001. In addition, it was significantly higher in those with active lesions (9.06) than those with inactive lesions (6.76); p-value was < 0.001 (Table 2).

The prediction of alopecia areata was performed using multivariate logistic regression analysis. It revealed that claudin was a significant predictor (OR = 1.217, 95% CI = 1.003–1.476, p-value = 0.046), controlling for the effect of age, sex, and smoking (Table 3).

4 | DISCUSSION

Alopecia areata is an immunological disorder that causes non-scarring hair loss in any hair-bearing area. It is considered to be a T-cell-mediated autoimmunity occurring in genetically predisposed individuals.[11] Alopecia areata's pathophysiology is not completely understood. But many research show that alopecia areata is linked to systemic autoimmune activation, which implies markedly increased serum levels of Th1 (IL-1, IL-2, IL-12, TNF-, and IFN-), Th2 (IL-4, IL-10, IL-13, IL-25, IL-31), and Th17 cytokines (e.g., IL-17A).[12] The involvement of short-chain fatty acids, primarily butyric acid, derived from microbial metabolism in the gut and the development of peripheral Treg cells has come to light, supporting the concept that there is a connection between AA and the gut microbiota.[13]

Claudins (CLDN) are tetraspan proteins consisting of a family of at least 24 members. It has been demonstrated that claudin-3 is expressed more strongly in distal than in proximal portions inside tubular tissues such as the intestine and nephron. It has been demonstrated that claudin-3 expression along rat intestinal segments correlates with barrier properties.[14] The relationship between the immune system, intestinal barrier, and gut microbiome has drawn a lot of attention. A significant contributor to the pathogenesis of psoriasis and a potential treatment target has been identified as the so-called gut-skin axis.[15]

We decided to evaluate the level of circulating claudin-3 in this study. Claudin-3 appears to be a viable potential indicator for identifying intestinal junctional damage because of its small size, transepithelial distribution, and widespread expression in the jejunum, ileum, and colon. Claudin-3 dysregulation has been observed as a sign of increased gut permeability in individuals with congenital heart disease, celiac disease, after strenuous exercise, and after major surgery.

In our study, the median claudin-3 was considerably higher in AA cases (7.73 ng/ml) compared to controls (6.14). Patients with AA experience anxiety and depression.[16] There have been reports of

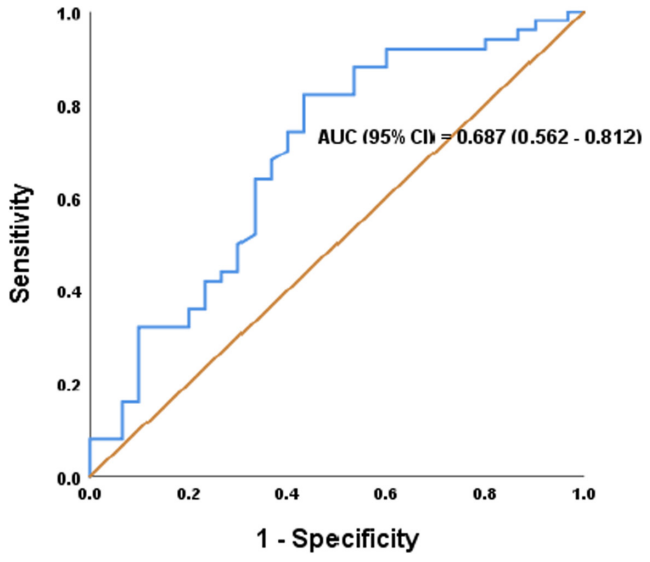

FIGURE 1 ROC analysis of claudin in diagnosing alopecia areata.

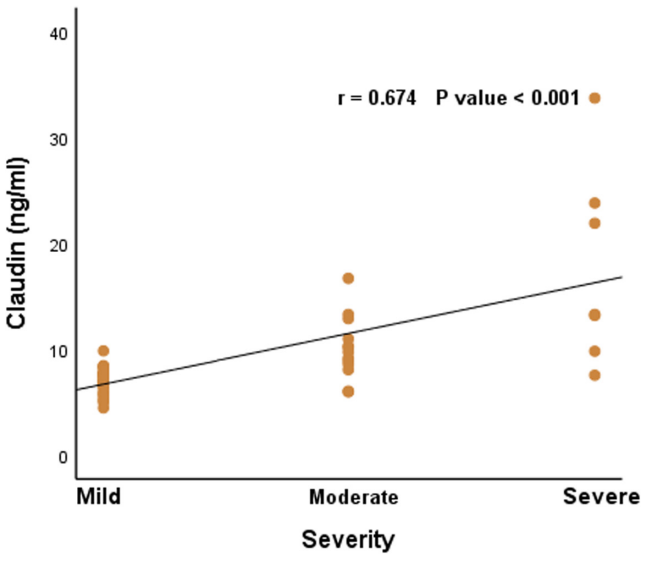

FIGURE 2 Correlation between claudin and severity.

TABLE 2 Claudin level according to different study parameters.

		Claudin (ng/ml)	*p*-Value
Sex	Males	7.89 (4.49–33.7)	0.172
	Females	7.32 (5–21.9)	
Smoking	Yes	7.64 (4.49–33.7)	0.873
	No	7.73 (5–21.9)	
Onset	Gradual	6.76 (5–9.18)	**0.034**
	Sudden	7.76 (4.49–33.7)	
Course	Stationary	7.74 (5–16.7)	0.582
	Progressive	7.69 (4.49–33.7)	
Recurrence	Yes	7.59 (4.49–33.7)	0.628
	No	7.81 (5.42–21.9)	
Number	Single	7.07 (4.49–11)	**<0.001**
	Multiple	9.48 (6–33.7)	
Activity	Active	9.06 (6.06–33.7)	**<0.001**
	Inactive	6.76 (4.49–9.86)	
Psychological stress	Yes	7.77 (4.49–33.7)	
	No	7.73 (5.21–21.9)	0.801
Associated disease	Yes	8.62 (5.21–33.7)	
	No	7.64 (4.49–23.8)	0.295
Family history	Yes	6.22 (5.8–8.93)	
	No	7.75 (4.49–33.7)	0.123

Note: Mann–Whitney U-test was used *p* Values ≤ 0.05.
Bold value indicates significant of *p* Values ≤ 0.05.

TABLE 3 Multivariate logistic regression analysis for prediction of alopecia areata.

	OR (95% CI)	P-value
Age (years)	1.023 (0.968–1.082)	0.419
Sex	0.952 (0.307–2.951)	0.932
Smoking	1.203 (0.329–4.397)	0.780
Claudin (ng/ml)	1.217 (1.003–1.476)	**0.046**

Abbreviations: 95% CI, 95% confidence interval *p* ≤0.05; OR, odds ratio.
Bold value indicates significant of *p* Values ≤ 0.05.

intestinal barrier disturbances in depressive disorders. Claudins are transmembrane proteins that interact with the actin cytoskeleton to create tight junctions. Loss of intestinal barrier function results from the knockout of particular claudins. Blood levels of claudin-3 are thought to be an effective indicator of intestinal permeability.[17] Gut permeability is strongly affected by changes in the microbiota.[18] We believe that this is the first study to examine the blood levels of claudin-3 in alopecia areata patients. The findings of this study suggest a potential connection between alopecia areata, the gut barrier, the microbiota, and the immune system.

We hypothesize that intestinal barrier degradation and gut immune response are both caused by alterations in the gut microbiota in autoimmune disease patients.[7] Antigens, bacteria, and their toxic compounds are more likely to enter the systemic circulation when the integrity and function of the intestinal barrier are compromised, which is made worse by local inflammation.[8] The mechanism promotes the development of a systemic inflammatory reaction, which in turn exacerbates disruption of the intestinal barrier. Our findings imply that interrupting the suggested vicious circle can enhance intestinal barrier performance. Our findings support the idea that one of the main targets for alopecia areata treatment plans could be intestinal permeability. The immune response is modulated by intestinal barrier disruption and subsequent bacterial metabolite translocation into the blood, which also affects other organs function including the skin. This serves as the basis for the idea of a "gut-skin axis."[19]

Our findings agreed with those of Sikora and colleagues (2019), who assessed claudin-3 in psoriasis, an autoimmune skin disease, and discovered that gut permeability, as measured by plasma claudin-3 concentrations, is enhanced in psoriasis patients.[15] High disease activity and smoking are linked to increased intestinal permeability. However, Watson and colleagues discovered that claudin-3 was not fully expressed in psoriatic skin independent of disease severity.[20]

In the current study, claudin-3 showed significant AUC (AUC = 0.853) with a 95% confidence interval ranging from 0.562 to 0.812. The best cutoff point was >6.37, at which sensitivity and specificity were 82% and 56.7%, respectively. To our knowledge, no other studies are available to compare with our findings.

The median claudin-3 was significantly higher in those with sudden onset (7.76 ng/ml) than those with gradual onset (6.76 ng/ml); *p*-value was 0.034. Also, it was significantly higher in those with multiple lesions (9.48) than those with single lesions (7.07); *p*-value

was < 0.001. Additionally, it was considerably higher in those with active lesions (9.06) than those with inactive lesions (6.76); *p*-value was < 0.001. Claudin-3 concentration significantly increased with increased AA severity. There is yet no recognized pathomechanism explaining the connection between the severity of AA and the loss of intestinal integrity as seen by the rise in claudin-3. We compared our results with a study[17] that measured claudin-3 in psoriasis as an autoimmune skin disease. The unique and crucial finding of Sikora and his colleagues study was a significant, positive connection between claudin-3 level and laboratory (neutrophil-to-lymphocyte ratio) and clinical (PASI) indicators of disease severity. In another recent study, PASI and claudin-3 levels were correlated.[14]

In the current study, alopecia areata was predicted using multivariate logistic regression analysis. It revealed that claudin-3 was a significant predictor (OR = 1.217, 95% CI = 1.003–1.476, *p*-value = 0.046) controlling for the effect of age, gender, and smoking.

5 | CONCLUSION

Alopecia areata patients had higher levels of claudin-3, an indicator for gastrointestinal permeability, which is correlated with disease severity.

AUTHOR CONTRIBUTIONS

Ihab Y. Abdallah, Hanaa E. Abd Elaal designated the research. Dina rafik M.B.B.Ch and Hanaa E. Abd Elaal collected the cases and performed the work. Rana A. Khashaba performed the biochemical work. Hanaa E. Abd Elaal MD[1] wrote the paper.

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This was an authors' own work. The manuscript has been read and approved by all the authors, and each author believes that the manuscript represents honest work. We are very grateful to all volunteers who took part in this study and the research team who collected the data.

CONFLICT OF INTEREST

The authors have declared that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ETHICAL APPROVAL

The work has been agreed by the Scientific Ethics Committee of Faculty of Medicine, according to Helsinki Declaration principles. Written informed consent was filled by subjects before being included in this study.

ORCID

Rana Atef https://orcid.org/0000-0003-2222-4382
Hanaa Eid https://orcid.org/0000-0002-0623-7987

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