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Assessment of Serum Level of Claudin- 3 and Its Association with Disease Severity in Patients with Psoriasis Vulgaris

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ABSTRACT

Background: Psoriasis is a common inflammatory disorder of the skin. Psoriasis is a disease of multifactorial origin where certain environmental factors acting on individuals with specific genetic predisposition leads to an immune dysregulation. Claudins are transmembrane proteins, which participate in the formation of tight junctions by binding to the actin cytoskeleton. Claudin- 3 present in the blood is considered as a useful biomarker of intestinal permeability.

Objective: To evaluate serum level of claudin-3 in patients with psoriasis in comparison to control group and correlate its levels with disease severity .

Patients and methods: Fifty-three patients (32 males and 21 females) with psoriasis and forty normal healthy control (23 males and 17 females) who matched the cases group as regard age and sex were included in this work. They were randomly selected from the Dermatology Department outpatient clinic in Mansoura University Hospital.

Results: Psoriasis group showed significantly higher level of claudin-3 when compared to control group (mean=58.3 versus 41.2; $p<0.001$). Smoking was significantly associated with higher Claudin-3 level ($p=0.031$). In addition, claudin-3 level increased gradually with increased severity grades ($p<0.001$). No significant associations were found regarding claudin-3 level according to gender, nutritional status, family history, in psoriasis group ($p>0.05$ for each). Higher BMI, smoking and higher claudin-3 level were associated with prediction of higher PASI score in univariate analysis. While multivariate analysis revealed that only smoking and higher claudin-3 level were considered independent predictor of more severe psoriasis cases.

Conclusion: Claudin-3 level was significantly higher in patients with psoriasis than healthy controls. PASI correlated with claudin-3 levels.

Keywords: Claudin- 3, PASI score, Psoriasis.

INTRODUCTION

Psoriasis is a disease of multifactorial origin where certain environmental factors acting on individuals with specific genetic predisposition lead to an immune dysregulation and abnormal keratinization, which results in the appearance of typical cutaneous lesions [(1)]. It is a chronic inflammatory skin disease with a genetic basis characterized by epidermal hyperproliferation, abnormal keratinocyte differentiation, T-lymphocyte infiltration, and increased expression of cytokines, which results in the formation of inflamed plaques [(2)]. Histologically, psoriasis is characterized by epidermal hyperproliferation with disordered keratinocyte differentiation, dermal inflammatory cell infiltration and increased vascularity [(3)].

Claudins (CLDN) are tetraspan proteins consisting of a family of at least 24 members. Within tubular structures like intestine and nephron, claudin-3 has been shown to be expressed stronger in distal than in proximal segments. It has been shown that expression of claudin-3 along segments of rat intestine correlates with barrier properties [(4)].

Recently, a considerable interest has been focused on interaction between gut microbiome, intestinal barrier and immune system. The so called gut skin axis has been considered as a key factor in the etiology of psoriasis and potential therapeutic target [(5)]. The intestinal epithelial barrier function consists of multiple defense mechanisms, which can basically be subdivided into a physical and an immunological barrier [(6)]. The physical intestinal barrier is composed of a lining of epithelial cells, connected by tight junctions [(7)].

Tight junctions are anchored in the cell via the filamentous actin (F-actin) cytoskeleton [(8)]. Zonula occludins proteins (ZO-1, ZO-2 and ZO-3) are important intracellular tight junction proteins, linking the cell cytoskeleton to the transmembrane tight junction proteins: claudins, occludin and junctional adhesion molecules (JAM). Whereas occludin and JAM have a regulatory role, claudins are transmembrane proteins mainly responsible for the intestinal barrier function [(9)].

Knockout of specific claudins leads to a loss of gut barrier function. Claudin-3 present in the blood is considered as a useful biomarker of intestinal permeability [(10)]. Physiological colonization of the gastrointestinal tract promotes intestinal barrier formation, whereas dysbiosis disrupts this process and contributes to the increased gut permeability. Translocation of bacteria, microbial toxins and metabolites into the peripheral circulation results in immune activation [(11)].

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Disturbances in intestinal barrier have been reported in metabolic and inflammatory comorbidities of psoriasis. So**,** claudin 3 level may be a clinically useful marker for gut permeability that is disturbed in psoriasis. Also, reinforcing intestinal barrier may be a new therapeutic target in psoriasis [(12)]**.**

The present study was carried out to evaluate serum level of claudin-3 in patients with psoriasis in comparison to control group and correlate its levels with disease severity.

PATIENTS AND METHODS

Ninety-three persons were included in this study, they were recruited from the out-patient clinic of Dermatology, Andrology and STDs Department, Mansoura University Hospitals in the period between March, 2020 and March, 2021. They were 53 patients and 40 healthy persons of matched age and sex who were included as a control group.

Ethical approval:

This study was approved by the Institutional Review Board (IRB) of Mansoura Faculty of Medicine (MS.20.02.1048). An informed consent was taken before inclusion of patients into the study. All precautions were taken to maintain the privacy of the data. All data were used for scientific purposes only.

Inclusion criteria: Patients with chronic plaque psoriasis above 18 years old.

Exclusion criteria: Patients receiving systemic therapy for psoriasis during last month prior to the study, age younger than 18 years, history of acute gastrointestinal infection during the last 3 months prior to the study, intake of antibiotics during the last 3 months prior to the study, dietary restrictions during the last 3 months prior to the study, history of gastrointestinal surgery during the last 6 months, liver cirrhosis, cardiac failure, and pregnancy and breastfeeding.

All patients underwent the following:

- **Detailed history taking** regarding age, sex, occupation, marital status, special habits, dietary intake, associated psychological disturbance, associated medical or surgical conditions and drug intake.
- **Detailed general examination:** all relevant clinical data were obtained e.g. height, body weight, body mass index (BMI), blood pressure.
- **Detailed dermatological examination** included skin, hair, nail and oral mucosa. Psoriasis vulgaris diagnosis criteria were pink to red scaly plaques and papules of variable size lesions that were dry and demarcated sharply usually covered with fine, silvery scales layers. When the scales were gently removed, scraping, tiny bleeding points i.e. Auspitz's sign, were found [(13)]**.**

Psoriasis area and severity index (PASI) score was calculated at enrollment, which includes a clinical assessment of psoriasis using the Psoriasis Area and Severity Index (PASI) score. PASI combines the assessment of the severity of lesions and the area affected into a single score in the range 0 (no disease) to 72 (maximal disease) [(14)]**.**

The body was divided into four sections (head (H) (10% of a person's skin); arms (A) (20%); trunk (T) (30%); legs (L) (40%)). Each of these areas was scored by itself, and then the four scores were combined into the final PASI. For each section, the percent of area of skin involved, was estimated and then transformed into a grade from 0 to 6: 0. 0% of involved area, 1. < 10% of involved area, 2. 10–29% of involved area, 3. 30–49% of involved area, 4. 50–69% of involved area, 5. 70–89% of involved area and 6. 90–100% of involved area.

Within each area, the severity was estimated by three clinical signs: erythema (redness), induration (thickness) and desquamation (scaling). Severity parameters were measured on a scale of 0 to 4, from none to maximum. The sum of all three severity parameters was then calculated for each section of skin, multiplied by the area score for that area and multiplied by weight of respective section (0.1 for head, 0.2 for arms, 0.3 for body and 0.4 for legs).

All patients underwent laboratory test for their lipid profile and serum level of claudin-3: 5 ml morning venous blood samples were collected from each person included in this study after an overnight 12- h fast. Blood was centrifuged, and sera were immediately separated and stored at −80 °C for further analysis. Assessment of serum claudin-3 was done by an ELISA technique as follow:

1. Prepare all reagents, standard solutions and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature.
2. Determine the number of strips required for the assay. Insert the strips in the frames for use. The unused strips should be stored at 2-8°C.
3. Add 5 µl standard to standard well. Note: Don't add antibody to standard well because the standard solution contains biotinylated antibody.
4. Add 40 µl sample to sample wells and then add 10 µl anti-CLND3 antibody to sample wells, then add 50 µl streptavidin-HRP to sample wells and standard wells (not blank control well) Mix well. Cover the plate with a sealer. Incubate 60 minutes at 37°C.
5. Remove the sealer and wash the plate 5 times with wash buffer. Soak wells with at least 0.35 ml wash buffer for 30 seconds to 1 minute for each wash. For automated washing aspirate all wells and wash 5 times with wash buffer, overfilling wells with wash buffer. Blot the plate onto paper towels or other absorbent material.

6. Add 50 µl substrate solution A to each well and then add 50 µl substrate solution B to each well. Incubate plate covered with a new sealer for 10 minutes at 37 °C in the dark.
7. Add 50 µl stop solution to each well, the blue color will change into yellow color immediately.
8. Determine the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

Statistical analysis and data interpretation:

The collected data were revised, coded, tabulated and introduced to a PC using Statistical Package for the Social Sciences (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.). Data were presented and suitable analysis was done according to the type of data obtained for each parameter. Mean, standard deviation (± SD) for parametric numerical data, median and range for nonparametric numerical data. Frequency and percentage of non-numerical data. Independent Student t test was used to assess the statistical significance of the difference between two study group means. Chi-square test was used to examine the relationship between two qualitative variables. The ROC Curve (receiver operating characteristic) was used to evaluate the sensitivity and specificity for quantitative diagnostic measures. Pearson's correlation coefficient was calculated. Regression analysis: Logistic and linear regression analyses were used for prediction of risk factors, using generalized linear models. P value was considered significant if <0.05.

RESULTS

Psoriasis cases were significantly associated with higher frequency of positive family history and smoking (Table 1).

Table (1): Comparison of demographic data, anthropometric data, history, blood pressure and lipid profile between cases and control groups

			Psoriasis N=53		Control N=40		p
Age (years)		**Mean±SD**	48.3	±5.5	44.2	±3.1	0.181
Males		**N, %**	32	60.4%	23	57.5%	0.780
Females		**N, %**	21	39.6%	17	42.5%	
Weight (kg)		**Mean±SD**	78.3	±6.1	74.4	±2.7	0.209
Height (m)		**Mean±SD**	1.7	±0.1	1.6	±0.1	<0.001
BMI (kg/cm^2)		**Mean±SD**	28.5	±6.2	28	±5	0.664
Nutritional status	**Non obese (BMI<30)**	**N, %**	28	52.8%	27	67.5%	0.154
	Obese (BMI≥30)	**N, %**	25	47.2%	13	32.5%	
Positive family history		**N, %**	10	18.9%	2	5%	**0.048**
Smoking		**N, %**	22	41.5%	4	10%	**0.001**
SBP (mmHg)		**Mean±SD**	120	±11.3	119.3	±8.6	0.726
DBP (mmHg)		**Mean±SD**	76.8	±6.4	76.3	±9.5	0.744
TC (mg/dL)		**Mean±SD**	166.8	±38.5	153.9	±24.1	0.066
TG (mg/dL)		**Mean±SD**	109.3	±3.4	106.6	±12.4	0.619
HDL (mg/dL)		**Mean±SD**	51.7	±9.3	59.8	±8.9	<0.001
LDL (mg/dL)		**Mean±SD**	93.3	±9.7	80.8	±4.0	0.192

SBP (systolic blood pressure); DBP (diastolic blood pressure). TC (total cholesterol)

Mean age of onset was 35.9 years old, mean disease duration was 12.4 years. Median baseline PASI score was 15.3 (Table 2). Disease severity cohorts were categorized based on PASI severity scores **Strober *et al.*** [(15)] into mild: 0 ≤ PASI ≤ 5; moderate: 5 < PASI ≤ 12; severe: 12 < PASI ≤ 20; very Severe: 20 < PASI.

Table (2): Clinical features in all studied Psoriasis cases

			Psoriasis (N=53)	
Age of onset (years)		**Mean±SD**	35.9	±11.8
Disease duration (years)		**Mean±SD**	12.4	±3
PASI		**Median (range)**	15.3	1.9-60
Grades	**Mild (1:9)**	**N, %**	4	7.5%
	Moderate (10:29)	**N, %**	17	32.1%
	Severe (30:49)	**N, %**	19	35.9%
	very severe (≥50)	**N, %**	13	24.5%

Psoriasis group showed significantly higher level of claudin-3 when compared to control group (Table 3).

Table (3): Comparison of claudin-3 level between all studied groups

	Psoriasis (N=53)		Control (N=40)		P
	mean	±SD	mean	±SD	
Claudin-3 (ng/mL)	58.3	12.6	41.2	8.9	**<0.001**

Claudin-3 showed good AUC (AUC=0.863). Best cut off value was 46.9 (Table 4).

Table (4): Validity of Claudin-3 levels for discrimination between psoriasis cases and control groups

	Claudin-3
AUC	0.863
Cut off	46.9
Sensitivity (%)	81.1
Specificity (%)	80
PPV (%)	84.3
NPV (%)	76.2
Accuracy (%)	80.6

AUC, area under curve; ROC, receiver operating curve; PPV, positive predictive value; NPV, negative predictive value.

Claudin-3 showed good AUC (AUC=0.853). The best cut off value was 54.9 (Table 5).

Table (5): AUC and performance features of Claudin-3 levels for discrimination of severe cases

	Claudin-3
AUC	0.853
Cut off	54.9
Sensitivity (%)	75
Specificity (%)	90.5
PPV (%)	92.3
NPV (%)	70.4
Accuracy (%)	81.1

AUC, area under ROC, OC, receiver operating curve; PPV, positive predictive value; NPV, negative predictive value.

Claudin-3 level showed significant positive correlation with PASI score (Table 6).

Table (6): Correlations of claudin-3 with age, BMI, BP, onset, duration and PASI score in psoriasis group

	Claudin-3	
	r	p
Age	-0.086	0.539
BMI	0.211	0.129
SBP	0.115	0.411
DBP	0.084	0.552
TC	0.006	0.965
TG	0.083	0.552
HDL	-0.088	0.530
LDL	0.009	0.950
onset	-0.166	0.234
duration	0.133	0.343
PASI score	0.782	**<0.001**

Higher BMI, smoking and higher Claudin-3 level were associated with prediction of higher PASI score in univariate analysis. While multivariate analysis revealed that only smoking and higher Claudin-3 level were considered independent predictor of more severe psoriasis cases (Table 7).

Table (7): Regression analysis for prediction of factors affecting severity of psoriasis (higher baseline PASI)

	Univariate		Multivariate	
	β	p	β	p
Age	-0.029	0.790		
Gender	2.535	0.144		
BMI	0.570	**0.028**	0.227	0.155
Positive family history	-2.117	0.615		
Smoking	2.564	**<0.001**	1.183	**0.010**
Onset	0.117	0.231		
Duration	0.217	0.144		
Claudin-3	0.753	**<0.001**	0.669	**<0.001**

B, linear regression coefficient.

DISCUSSION

The present study was carried out to evaluate serum level of claudin-3 in patients with psoriasis in comparison to control group. In our study, the mean age of psoriasis group was 48.3 years, they were 32 males (60.4%) and 21 females (39.6%). Cases and control groups had matched age and gender. This mean age was in accordance with **Megna *et al.*** [16] **and El-Hanafy *et al.*** [17]**. Icen *et al.*** [18] who found that the mean age at diagnosis of psoriasis (± standard deviation) was 43.2 ± 17.0 years and 828 (51.0%) subjects were male. The male: female ratio in our patient group was 32:21. Similarly, **Mala *et al.*** [19] found that the male: female ratio in patients group was 21:19. However, **Burns *et al.*** [20] noticed that both genders were equally affected.

Weight, height, BMI, nutritional status, blood pressure did not show significant differences between both groups. In our study, mean BMI in patient and control groups was 28.5±6.2 and 28±5, respectively with no statistically significant difference. Similarly, a comparison between 200 patients with psoriasis diagnosed within the previous 12 months and matched controls showed no statistical difference in BMI between both groups [21]**.** Also, our results came in agreement with the results of **Pietrzak *et al*** [22]**.** This supports the finding that obesity occurs at some point after the manifestation of psoriasis. Mechanisms by which this occurs emphasize the frequent self-perceived cosmetic disfigurement caused by psoriasis, resulting in social isolation, unhealthy nutrition habits, depression, and alcohol consumption [23]**.**

Regarding blood pressure, the mean SBP in patient and control groups was 119.3 and 120 respectively with no statistically significant difference. Also, the same for DBP. In the study of **Praveenkumar *et al.*** [24]**,** they observed elevated blood pressure; systolic ≥130 mm of Hg and/or diastolic ≥ 85 mm of Hg, as a criterion of metabolic syndrome was found in equal percentage of cases and controls. In contrast, **Cohen *et al.*** [25] found that prevalence of hypertension was significantly higher in psoriasis patients than controls. Hypertension was associated with psoriasis after controlling for age, sex, smoking status, obesity, diabetes, non-steroidal anti-inflammatory drugs and use of Cox-2 inhibitors.

In the present study, psoriasis cases were significantly associated with higher frequency of positive family history and smoking. There was a statistically significant difference in family history of psoriasis in patients versus controls in our study similar to published data by **Solmaz *et al.*** [26] who proved that psoriasis and psoriatic arthritis (PsA) are diseases that have strong genetic backgrounds. The role of genetics is not only important for the occurrence of the disease but also impacts the phenotype of the patients. There was a statistically significant difference in smoking frequency in patients versus controls in our study similar to published data in **Naldi** [27] who stated that not only has smoking been associated with the onset of psoriasis but it has also been linked with the severity of the disease and response to treatment. This was in disagreement with a retrospective study of 66 psoriatic patients that did not show any significant difference between smokers and nonsmokers in terms of clinical improvement in cutaneous psoriasis among on various systemic treatment [28]**.** Also, the percentage of subjects with history of smoking among patients and healthy controls did not differ significantly in **Praveenkumar *et al.*** [24] study.

In the present study, we did not find any significant differences between cases and controls regarding lipid profile (TC, TG, HDL and LDL). The correlation between dyslipidemia and psoriasis is variable in different studies. Our results were in agreement with the results of **Kim *et al.*** [29] who reported that there was not any significant difference in the prevalence of dyslipidemia between the psoriasis and control groups after controlling for age and gender (p=0.200). **Lakshmi *et al.*** [30] showed a higher average triglyceride level among the patients compared to controls, even though it was not statistically significant. That study also showed

paradoxically higher average HDL level among psoriasis patients compared to controls. The lack of statistical significance in our study can be explained by our small sample size. Since this is a novel finding of lipid profile, it needs to be investigated further by large population-based studies as well as experimental studies to elucidate the molecular etiopathogenetic links.

In our study mean age of onset was 35.9 years old. Psoriasis onset may occur at any age; however, some studies have demonstrated a bimodal distribution of age of onset in both male and female patients with psoriasis. One peak occurred at age 16 years for females and 22 years for males, with another peak at age 60 years for females and 57 years for males [31]. Two subtypes of psoriasis have been identified based on age of onset: early-onset psoriasis (EOP; age≤40 years) and late-onset psoriasis (LOP; age>40 years). The dichotomizing of psoriasis patients into early versus late onset of disease is consistent with differences in genetic predisposition and clinical presentation [32].

In our study, PASI scores ranged from 1.9 to 60, and the mean PASI score was 15.3. In **Yavuz Daglioglu *et al.*** [33] study, PASI scores ranged from 0.9 to 48, and the mean PASI score was 14.34±10.13. Also in **Melikoglu** [34] study the mean PASI was 14.1±5.3.

In the current study, claudin-3 showed good AUC (AUC=0.853). At best cut off value of 54.9, sensitivity was 75%, specificity was 90.5%, PPV was 92.3%, NPV was 70.4%, and accuracy was 81.1%. To our knowledge no more available studies for comparing our results.

In the current study, smoking was significantly associated with higher claudin-3 level. In addition, claudin-3 level increased gradually with increased severity grades. A multivariate linear regression analysis conducted by **Sikora *et al.*** [35] confirmed association of claudin- 3 with the PASI score ($P < 0.001$) and active smoking ($P < 0.05$).

Interestingly, no significant associations were found by us regarding Claudin-3 level according to gender, nutritional status, family history, in our psoriasis group.

In the current study, claudin-3 level showed significant positive correlation with PASI score but not with age, BMI, BP, lipid profile, onset, duration. Similarly, **Sikora *et al.*** [35] found positive correlation between claudin- 3 concentration and the PASI score ($r = 0.828$; $P < 0.001$) also no association with BMI was found ($r = 0.099$; $P = 0.45$).

Logistic regression analysis was conducted by us for prediction of psoriasis development using age, gender, BMI, family history, smoking and claudin-3 level as confounders. Positive family history, smoking and higher claudin-3 level were significantly associated with prediction of psoriasis development in univariate analysis. However, taking significant covariates in univariate analysis into multivariate analysis revealed that only higher claudin-3 level was considered independent predictor of psoriasis development.

A multivariate linear regression analysis was carried out by **Sikora *et al.*** [35]**.** They introduced all variables into the analysis, the PASI score (β coefficient: 0.608; $P < 0.001$) and smoking (β coefficient: 0.165; $P < 0.05$) were independently associated with higher claudin- 3 concentration in patients with psoriasis. There were no statistically significant associations with other clinical parameters (age, sex, BMI, and lipid profile).

CONCLUSION

Claudin-3 level was significantly higher in patients with psoriasis than healthy controls. PASI correlated with claudin-3 levels. Smoking and higher baseline claudin-3 level were suggested to be independent risk predictor for psoriasis occurrence and severity.

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