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Evaluation of Serum Level of Calprotectin in Patients with Psoriasis and Its Relation to The Clinical Severity of The Disease

Amr Mohamed Zaki[1], Mohamed Abdelmawgoud Amer[1], Nagah Mohamed A. Mohamed[2], Mohamed Ahmed El-saeed Abdelkhalik[1*]

Departments of [1]Dermatology & Venereology and [2]Clinical Pathology & Immunology, Faculty of Medicine, Al-Azhar University

***Correspondence author:** Mohamed Ahmed El-saeed Abdelkhalik, **Mobile:** (+20) 01016873338, **E-Mail:** mohamedshazly1135@gmail.com

ABSTRACT

Background: Psoriasis is a common chronic immune mediated papulosquamous disease. Its prevalence around 2-3% of the general population,and is characterized by an exaggerated proliferation of keratinocytes secondary to an activated immune system. **Objective:** the aim of this work was to investigate the relationship between serum Calprotectin and psoriasis vulgaris and to correlate with disease severity.

Patients and Methods: the present study was conducted on 50 patients with psoriasis (group A) and 30 healthy control subjects (group B). Both groups were subjected to full history taking, clinical examination and estimation of serum level of calprotectin using ELISA technique. PASI score was used to assess disease severity in group A.

Results: calprotectin level was significantly higher in cases group than in control group. A positive statistical correlation between the calprotectin level and the disease severity (PASI score) was observed but not with age, sex, nor duration of disease. **Conclusion:** calprotectin can be used as a marker of psoriasis severity and progression. Calprotectin may play a role in the pathogenesis of psoriasis.

Keywords: Calprotectin, Psoriasis, autoimmune diseases.

INTRODUCTION

Plaque-type psoriasis is the most common form, affecting 80 – 90% of patients. Patients present with sharply demarcated, erythematous plaques covered by silvery white scales, most commonly on the extensor surfaces and the scalp. Patients may experience extracutaneous manifestations commonly including nail involvement and psoriatic arthritis in up to 20% of patients [(1)]. The genetic, immunological and environmental factors contribute to the pathogenesis of psoriasis. However, its precise etiology is not yet fully elucidated [(2)].

In the last decades, S100 proteins have been increasingly emerging as a key player of innate immunity, important in the pathogenesis of various inflammatory, metabolic, and neoplastic disorders. They are produced as monomers and spontaneously form dimers/multimers[(3)].

S100A9 together with S100A8 proteins forms a heterodimeric complex, termed calprotectin[(4)].

Calprotectin is a calcium-binding cytosolic protein that belongs to theS100 family, which presents in regenerative cellssuch as neutrophils, monocytes, macrophages, epithelial, and endothelial cells [(5)]. It was suggested that this protein might be a promising marker of inflammation [(6)].

Calprotectin is related to disease activity in several inflammatory and autoimmune diseases, such as: SLE [(7)], Rheumatoid arthritis [(8)], Still's disease[(9)], Acute gouty arthritis [(10)]. Fecal calprotectin has good diagnostic value to identify IBD and differentiates it from functional disorders of the gut. It can be used to detect disease activity, predict relapse, and monitor response to therapy in IBD[(11)]. Evidence is accumulating for a role of calprotectin in the pathogenesis of psoriasis, including a direct chemotactic effect on various immune cells involved in the induction of pro-inflammatory cytokines by keratinocytes and stimulation of pro-angiogenic mediator production also by keratinocytes[(12)].

AIM OF THE WORK

The aim of this work is to investigate the relationship between serum Calprotectin and psoriasis vulgaris and to correlate with disease severity.

PATIENTS AND METHODS

The present study was conducted at Dermatology outpatient clinics of Al-Azhar University Hospital, Elhousin university hospital. It included two groups as follow:

Group A (cases group): included 50 patients presented clinically with plaque psoriasis randomly selected from Dermatology outpatient clinics.

Group B (control group): included 30 healthy subjects as control.

Written informed consent:

An approval of the study was obtained from Al-Azhar University Academic and Ethical Committee. Every patient signed an informed written consent for acceptance of the operation.

Inclusion criteria: Cases with Psoriasis vulgaris.

Exclusion criteria:

- Patients on topical or systemic therapy for psoriasis during the last fourweeksprior to the study.
- Any apparent sign of acute or chronic inflammation (e.g., hepatitis, arthritis, inflammatory bowel diseases).
- Connective tissue diseases.
- Liver or renal impairment.
- Malignancies.
- Acute or chronic infections.

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- Any dermatological disease other than psoriasis.

All participants were subjected to the following:

a. **Full history taking:** Personal history. Present history of the condition. Drug history. & History taking of any other diseases.

I. Examination:

a) General medical examination.

b) Dermatological examination:

- Patients were examined to determine: site, size, distribution, number of lesions.
- The psoriasis area and severity index (PASI) score was measured as regards; erythema, thickness, scaling and surface area involved and both intensity and extent of the psoriatic plaques were calculated[13].
- Four anatomical regions were assessed:
  - Head (h)
  - Trunk (t)
  - Upper extremities (u)
  - Lower extremities (l).

The areas of psosriatic involvement of these four main areas (Ah, At, Au, Al) respectively were given a numerical value (from 0 to 6):

0 = no involvement,
1 = 1–9%,
2 = 10–29%,
3 = 30–49%,
4 = 50–69%,
5 = 70–89% and
6 = 90–100% body surface area (BSA) involvement.

The intensity of erythema (E), thickness or infiltration (I) and scales or desquamation (D) was rated on a 5-points scale (from 0 to 4) with:

0 = no involvement,
1 = slight,
2 = moderate,
3 = severe and,
4 = very severe characteristics.

The percentage of involvement of the four anatomical regions is assigned a numerical value of 0–6 with:

Values entered into the formula:

0.1(Eh + Ih + Dh)Ah + 0.2(Eu + Iu + Du)Au + 0.3(Et + It + Dt)At + 0.4(El + Il + Dl)Al to calculate a score from 0 to 72where 0 represents "no possible lesionat all" and 72 represents complete erythroderma of the severe possible degree[14].

II. Laboratory Investigations:

Calprotectin level:

Serum levels of calprotectin were quantitated with commercially available enzyme linked immunosorbent assay (ELISA) kit (Bioassay Technology Laboratory; Cat. No E4010Hu).

Sampling:

Five ml of venous blood were collected from the patients and from the healthy individuals. The serum was separated by centrifugation and stored at -70 °C until samples of patients and control groups were assayed in one run.

Principle of the method:

Calprotectin (CAL) was added to the wells pre-coated with CAL monoclonal antibody. After cubation a biotin-conjugated anti-human CAL antibody was added and binds to human CAL. After incubation unbound biotin-conjugated anti-human CAL antibody was washed away during a washing step. Streptavidin-HRP was added and binds to the biotin-conjugated anti-human CAL antibody. After incubation unbound Streptavidin-HRP was washed away during a washing step. Substrate solution was then added and color developed in proportion to the amount of human CAL. The reaction was terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

The assay was performed in a blind fashion on coded samples by an investigator who was not informed of the subject's clinical status, after the collection of all samples had been completed.

Statistical analysis

The collected data were coded, processed and analyzed using the SPSS (Statistical Package for Social Sciences) version 15 for Windows® (SPSS Inc, Chicago, IL, USA). Qualitative data was presented as number and percent. Comparison between groups was done by Chi-Square test. Quantitative data was tested for normality by Kolmogrov-Smirnov test. Normally distributed data was presented as mean ± SD. Non parametric data was presented as min – max and median. Mann-Whitney test was used for comparison between groups. Kruskal-Wallis test was used to compare between more than two groups. Spearman's correlation coefficient was used to test correlation between variables. $P < 0.05$ was considered to be statistically significant.

RESULTS

Demographic data:

Group A included 28 males (56%) and 22 females (44%). Their ages ranged from 8 years to 67 years with mean age of 38.02±17.30 years. Group B included 20 males (66.7%) and 10 females (33.3 %). Their ages ranged from 17 years to 55 years with mean age of 32.30±11.43 years. There was no statistically significant difference between the two groups regarding sex (p=0.346) and age (p=0.079) (Table 1, 2).

Table (1): Comparison between the two studied groups according to sex

Sex	Patients (n = 50)		Control (n = 30)		χ2	P
	No	%	No	%		
Male	28	56%	20	66.7%	0.889	0.346
Female	22	44%	10	33.3%		

Table (2): Comparison between the two studied groups according to age

Age	Patients (n = 50)	Control (n = 30)	t	P
Mean ± SD	38.02 ± 17.30	32.30 ± 11.43	1.779	0.079
Median	37.5	30.5		

Evaluation of calprotectin level:

On evaluation of calprotectin level, the mean level (Mean ± SD) among psoriasis patients (n=50) was 141.34±177.47 ng/ml, and among control subjects (n=30) was 40.03±31.54 ng/ml. The Median was 68.5 among psoriasis patients and was 35 among control subjects. A statistically significant lower level was observed in control group than in cases group (P <0.05) (Table 3).

Table (3): Comparison between the two studied groups according to calprotectin level (ng/ml)

S.Calprotectin	Patients (n = 50)	Control (n = 30)	Z	P
Mean ± SD	141.34 ± 17.47	40.03 ± 1.54		
Median	68.5	35	3.360	0.001*

*

Significant P < 0.05

Relations and correlations with calprotectin:

On comparing the serum level of calprotectin among males and females, there was no statistically significant difference of calprotectin level between males and females among psoriasis patients (p=0.551) and also no significant difference of calprotectin level between males and females among controls (p=0.547) (Table 4).

Table (4): Relation between sex and calprotectin in cases (group A)

Patients	Male (n = 28)	Female (n = 22)	Z	P
S.Calprotectin				
Mean ± SD	143.93 ± 20.12	138.05 ± 14.92		
Median	66	71	0.596	0.551

Table (5): Relation between sex and calprotectin in control (group B)

Control	Male (n = 20)	Female (n = 10)	Z	P
S. Calprotectin				
Mean ± SD	37.50 ± 1.29	45.10 ± 3.08		
Median	29.5	41.5	0.484	0.628

There is significant difference of calprotectin level (p=0.007) between mild PASI which has Mean calprotectin level (Mean ± SD) of 88.70 ± 120.83 , moderate PASI which has Mean calprotectin level (Mean ± SD) of 134.63 ± 145.71 and severe PASI which has Mean calprotectin level (Mean ± SD) of 308.63 ± 280.68 among psoriasis patients (Table 6).

Table (6): Relation between PASI and calprotectin in cases (group A)

S. Calprotectin	PASI Score		
	Mild (n = 23)	Moderate (n = 19)	Severe (n = 8)
Mean ± SD	88.70 ± 120.83	134.63 ± 145.71	308.63 ± 280.68
Median	45	79	274.5
$\chi 2$	9.789		
P	0.007*		

* Significant P < 0.05

There was no statistically significant difference detected between different disease durations as regards the calprotectin level (p=0.335) (Table 7).

Table (7): Relation between disease duration and calprotectin in cases (group A)

S. Calprotectin	Disease Duration		
	<5y (n = 24)	6-15y (n = 17)	>15y (n = 9)
Mean ± SD	108.58 ± 140.42	129.88 ± 118.98	250.33 ± 301.74
Median	51	87	112
$\chi 2$	2.188		
P	0.335		

No significant correlation between calprotectin level and different age groups was detected in patients (p=0.136). There is also no significant correlation between calprotectin level and different disease durations was detected (p=0.310). There is significant positive correlation between calprotectin level and different PASI scores was detected in patients (p<0.001) (Table 8).

Table (8): Correlation between calprotectin with different parameters in cases (group A)

	S. Calprotectin	
	r	P
Age	0.214	0.136
Duration of Disease	0.147	0.310
PASI score	0.547	<0.001*

* Significant P < 0.05

DISCUSSION

Studies measuring the calprotectin levels in patients with psoriasis are considered to be insufficient. Garcia-Rodriguez et al. established high levels of plasma calprotectin in psoriatic patients; however, this result was not statistically significant [(15)].

The aim of this study was to measure the serum level of calprotectin in chronic psoriatic patients, comparing them with healthy controls and correlate with disease severity.

The present study was conducted on 50 patients with psoriasis (group A) and 30 healthy control subjects (group B). Both groups were subjected to full history taking, clinical examination and estimation of serum level of calprotectin using ELISA technique. PASI score was used to assess disease severity in group A[(13)].

Group A included 28 males and 22 females, their ages ranged from 8 years to 67. Group B included 20 males and 10 females, their ages ranged from 17 years to 55 years. There was no statistically significant difference between the two groups regarding sex and age.

Regarding calprotectin, the mean serum level was significantly higher in psoriatic patients than the control subject.

Guzel *et al.*[(16)], Aochi *et al.*[(17)] and **Benoit *et al.*[(18)]** found significantly higher levels of serum calprotectin in patients with psoriasis compared with a control group, and these results goes hand in hand with the results of the present study.

In the present study, the relation between PASI score and level of calprotectin showed that, mean calprotectin level of patients with severe PASI was significantly higher than mean calprotectin level of patients with moderate PASI which was higher than mean calprotectin level of patients with mild PASI. A significant positive correlation was observed between PASI score and level of calprotectin.

Similar results, regarding increased serum levels of calprotectin in psoriatic patients than healthy control subjects and its correlation with disease severity according to PASI score, were reported by several studies [(16, 18, 19)].

However, in the present study the mean serum levels of calprotectin in psoriatic patients were lower than those reported by **Guzel *et al.*[(16)]** and **Qian and Song[(19)]**. This could be attributed to racial factors.

In the present study, the disease duration was classified to 3 categories **Huang *et al.*[(20)]** (≤ 5 years, between 6 and 15 years and ≥15 years) and disease duration ≤ 5 years was reported in 48% of cases (24 patients), between 6 and15 years in 34% of cases (17 patients), and more than 15 years in 18% of cases (9 cases).

Regarding disease duration, the present study revealed that there was no statistically significant difference between disease duration and calprotectin level. In other words, it seems that calprotectin is not a clue for disease duration.

Also no significant correlation was found between levels of calprotectin and age or sex among psoriatic patients.

The present study and studies on calprotectin in psoriasis had shed the light on the suggested role of calprotectin in the pathogenesis of psoriasis. Calprotectin serum levels were found to be elevated in psoriatic patients. Moreover, elevated calprotectin serum levels correlated with the activity of psoriasis,

therefore S100 proteins could be considered as potential mediators in psoriasis.

CONCLUSION

- Calprotectin level was significantly higher in cases group than in control group
- A significant positive correlation between PASI score and level of calprotectin. The serum calprotectin level increased as the severity of psoriasis increased.
- Calprotectin can be used as a marker of psoriasis severity and progression.
- Calprotectin may play a role in the pathogenesis of psoriasis.

RECOMMENDATIONS

According to the current results we recommend to:

- Have further studies of calprotectin level in psoriatic population on larger number of patients.
- Use Calprotectin as an investigation in psoriatic patient for better therapeutic approaches.
- Have further studies to compare calprotectin with other inflammatory markers of psoriasis in order to prove its clinical value.

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