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ORIGINAL ARTICLE

Role of Serum and Urinary Orsomucoid Protein A in Psoriatic Arthritis Patients and Their Relation to Disease Activity

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ABSTRACT

Background: The musculoskeletal disorder known as psoriatic arthritis (PsA) is heterogeneous and can cause a range of symptoms, such as axial involvement, dactylitis, enthesitis, and arthritis. For as many as 30% of patients, psoriasis or nail disease may coexist with musculoskeletal complaints. Serum and urine orsomucoid (ORM) levels have been shown to have an inflammatory role in psoriasis, making them one of the few biomarkers associated with the disease. However, no research has been done on PsA patients; therefore, our goal was to evaluate the levels of ORM in PsA patients' serum and urine and their relationship to disease activity.

Methods: Subjects who participated in this case-control study were collected from the Rheumatology and Rehabilitation Department at Zagazig University Hospitals. They were divided into two equal groups (control and PsA). The Disease Activity Index for Psoriatic Arthritis (DAPSA) was used to measure PsA activity, while the Psoriasis Area and Severity Index (PASI) was used to measure skin severity. Plain X-ray oblique view and MRI by STIR technique on the sacroiliac joint were done. Serum, urinary orsomucoid protein A, and detection of orsomucoid urine/creatinine urine ratio by Human Orsomucoid ELISA were done.

Results: PsA had considerably greater serum and urine orsomucoid protein A levels and urine orsomucoid/creatinine ratios than controls. Urine orsomucoid and disease activity showed a strong positive connection.

Conclusions: Orsomucoid may be a useful biomarker for psoriatic arthritis patients to assess the disease activity and may play a role in the pathophysiology of the condition.

Keywords: Psoriatic; Orsomucoid; Activity; Arthritis.

INTRODUCTION

The musculoskeletal disorder known as psoriatic arthritis (PsA) is heterogeneous and can cause a range of symptoms, such as axial involvement, dactylitis, enthesitis, and arthritis. Up to 30% of individuals also have concurrent nail diseases or psoriasis in addition to musculoskeletal complaints. The quality of life (QoL) may be significantly impacted by both psoriasis and psoriatic arthritis. Health-related QoL is particularly significantly impacted by pain and functional impairment [1]. Because PsA patients typically appear with a variety of clinical manifestations, diagnosis and therapy are sometimes postponed or overlooked entirely. More recently, studies have sought to discover new disease biomarkers in order to enhance PsA diagnosis and prognosis [2]. Acute phase protein (APP) called orsomucoid (ORM) is mostly produced in the liver. ORM is predominantly synthesized by hepatocytes and parenchymal cells upon stimulation by proinflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin (IL)-1, and IL-6, which stimulate the production of ORM [3].

In the past, autoimmune disorders such as rheumatoid arthritis, systemic lupus erythematosus, inflammatory bowel disease, multiple sclerosis, and adult-onset Still's disease were diagnosed and tracked using this protein. Additionally, high levels of this protein increase the incidence of infections, cancer, and diabetes mellitus [4]. For the best possible care and early detection of consequences, disease activity must be monitored. This is the first study to measure orsomucoid protein A levels in the urine and serum of Egyptian individuals with psoriatic arthritis.

METHODS

This case-control study was conducted at the Rheumatology and Rehabilitation Department of the Faculty of Medicine, Zagazig University Hospitals. A total of 30 PsA patients, diagnosed based on the CASPAR criteria classification [5], and 30 healthy age- and sex-matched controls were included. Patients with other autoimmune diseases, acute inflammation, impaired renal function, infections, and malignancies were excluded.

Clinical and radiological evaluation

Every patient underwent a clinical evaluation, including a musculoskeletal and general assessment. The Disease Activity Index for Psoriatic Arthritis (DAPSA) was utilized to evaluate the disease activity of psoriatic arthritis (0-4 remission, 5-14 low disease activity, 15-28 moderate disease activity, >28 high disease activity) [6]. The Psoriasis Area and Severity Index (PASI) was used to measure the severity of skin affection [7]. Plain X-ray and MRI on the sacroiliac joint were done.

Laboratory investigations

Blood and urine samples had been thoroughly analyzed at the Clinical Pathology Department. Two ml of blood were drawn into a BD vacutainer® ESR tube, three ml into a BD vacutainer, and two ml into an EDTA tube (Becton, Dickinson and Company, NJ). Blood samples were centrifuged at 1200xg for 10 minutes after being allowed to clot at room temperature for 30 minutes in order to separate the serum. A portion of the serum was isolated and refrigerated at -80°C in preparation for later evaluation using a human orsomucoid ELISA. Urine samples were centrifuged, the supernatant separated, and preserved at –80°C till use for Human Orsomucoid ELISA later. Other laboratory tests that were performed included complete blood count by fully automated cell counter (XN 1000 Sysmex, Germany), erythrocyte sedimentation rate (ESR) using the automated ESR analyzer Vision B (Shenzhen, YHLO Biotech Co., Ltd., Shenzhen, China), C-reactive protein (CRP) using the Cobas 6000, c501 module (Roche Diagnostics, Mannheim, Germany) that uses an immunoturbidimetry assay, serum and urine creatinine using the Cobas 8000, c501 module (Roche diagnostics, Mannheim, Germany), and liver function tests.

Measurement of serum and urine orsomucoid protein A levels

Measurement of serum and urinary orsomucoid protein A levels and detection of urine orsomucoid/ urine creatinine ratio were done by Human Orsomucoid ELISA Kit ‘Catalog No: DLR-a1AGP-Hu (DL-develop Co, China). As directed by the manufacturer, the assay was carried out. A standard curve was used to calculate the Human Orsomucoid levels. The findings were translated as mg/L for urine and ng/mL for serum. Wells were identified for the sample, blank, and diluted standard. Two blank wells and fourteen wells for the standards were prepared because every test was done in duplicate. Subsequently, 100 µL dilutions of the standard, blank, and samples were introduced into the corresponding wells. Following the Plate Sealer's application, the plate was incubated at 37°C for 90 minutes. Following the addition of 100 µL of Detection Solution A, each well was covered with the Plate sealer and incubated for 45 minutes at 37°C. After aspirating the solution, 300 µL of 1× Wash Solution was added, and it was allowed to settle for 1-2 minutes. All of the liquid was extracted by tapping the plate into absorbent paper in each well. After that, it had three full washings. Aspiration was used to remove any leftover Wash Buffer following the final wash. Subsequently, the plate was turned over and blotted onto absorbent paper. After covering the plate with the plate sealer, 100 µL of Detection Solution B was poured into each well, and it was then incubated for 45 minutes at 37°C. The aspiration/wash procedure was done five times in total. Subsequently, 90 µL of substrate solution was added to each well (the addition of substrate solution caused the liquid to turn blue). After covering the plate with a fresh Plate Sealer, it was kept out of the light and incubated for 20 minutes at 37°C. Ultimately, each well received 50 µL of stop solution, which caused the liquid to turn yellow. Next, the liquid was combined by tapping the plate's side. Water drops and fingerprints from the plate's bottom were eliminated, and it was determined that there were no bubbles in the liquid's surface. Microplate reader utilized for instantaneous absorbance measurements at 450 nm. Plotting the mean O.D. and concentration for each standard

allowed us to construct a standard curve, which we then either created on log-log graph paper with absorbance on the x-axis and Human Orsomucoid concentration on the y-axis, or we drew a best-fit curve across the graph's points.

Sample size calculation

Sample size was calculated by assuming the mean ORM was 0.45±0.43 vs. 0.21±0.17 in cases vs. control, respectively. At 80% power and 95% CI, the estimated sample will be 60 subjects; 30 subjects in each group using Open Epi.

Ethical considerations

The Institutional Review Board (IRB number: 11091/9/2023) at the Faculty of Medicine, Zagazig University, granted ethical approval. Participants signed a written informed consent before enrollment in this study. The study was conducted in compliance with the Declaration of Helsinki, the World Medical Association's code of ethics for human subjects' research.

Statistical analysis

Version 26 of the SPSS (Statistical Package for the Social Sciences) program was used to analyze the data. The Chi-square and Fisher's exact tests were used to compare categorical variables, and their absolute frequencies were used to describe them. The Shapiro-Wilk test was employed to confirm the assumptions made for parametric testing. Depending on the type of data, the mean and standard deviation (SD) or the median and interquartile range (IQR) were used to characterize quantitative variables. The independent sample t-test (for normally distributed data) and the Mann-Whitney test (for non-normally distributed data) were used to compare quantitative data between two groups. The one-way ANOVA test was performed for normally distributed data, and the Kruskal-Wallis test was employed for data that was not normally distributed. Pairwise comparison and the Bonferroni test were used to identify differences between each of the two individual groups where the differences were significant. In order to diagnose a certain health issue, the ROC curve was utilized to find the best cutoff of a particular quantitative parameter. The direction and strength of the link between two variables were evaluated using the Spearman rank correlation coefficient. To measure related independent components for the dependent factor, linear regression analysis was done. $P < 0.05$ was chosen as the level of statistical significance. If $P \leq 0.001$, a highly significant difference was detected.

RESULTS

The age, sex, and body mass index (BMI) of the two groups did not significantly differ from one another. Females were 50% in PsA patients and 73.3% in the control group. The mean age of the patients was 44.03 ± 12.4 years, while the control group's mean age was 37.3 ± 14.31 years (P = 0.056). Patients and controls had mean BMIs of 27.2 ± 3.67 and 28.2 ± 1.7, respectively (P = 0.395). The frequency of nail changes, dactylitis, enthesopathy, and sacroiliitis was 76.7%, 16.7%, 63.3%, and 50% of patients, respectively. There were 1 to 63 sore joints in all. The range of swollen joints was 0 to 10. The PASI score ranged from 0.3 to 66.6 with a median of 10.05. The DAPSA score ranged from 3.6 to 96.15 with a median of 48. Laboratory examinations revealed a significantly higher ESR, CRP, serum orsomucoid, urine orsomucoid, and urinary orsomucoid to urinary creatinine ratio in PsA patients compared to the control group (P=0.021, P=0.004, P=0.007, P <0.001, and P <0.001, respectively) (Table 1).

PsA patients were subgrouped, according to disease activity assessment using the DAPSA, into remission to low (5.17-14) and moderate to high (45-73) groups. There was a statistically significant correlation between the existence of enthesopathy, sacroiliitis, DAPSA score, levels of ESR, and CRP, and disease activity (Table 2).

Urine orsomucoid and the number of sore joints and DAPSA score showed a substantial positive correlation (P <0.001, P <0.001, respectively) (Table 3).

According to ROC curve analysis, for the diagnosis of PsA, a cutoff value of serum orsomucoid of ≥25.031 ng/ml with an area under the curve (AUC) of 0.704 demonstrated 70% sensitivity, 66.7% specificity, 67.7% positive predictive value (PPV), 69% negative predictive value (NPV), and 68.3% overall accuracy. The urine orsomucoid cutoff value was ≥0.306 mg/L, the AUC for this value was 0.936, and the sensitivity, specificity, PPV, NPV, and overall accuracy were all 86.7% (P ≤0.001) (Table 4, Figure 1).

Urine orsomucoid and DAPSA score had a substantial positive correlation (r=0.742 & P <0.001), as demonstrated by the scatter dot plot (Figure 2).

Table (1): Comparison between the studied groups regarding demographic, clinical and laboratory data

	PsA patients (n=30)		Control group (n=30)		χ^2	p
Sex, N (%) Female Male	 15 (50%) 15 (50%)		 22 (73.3%) 8 (26.7%)		3.455	0.063
	-		-		t	P
Age (year) Mean ± SD	44.03 ± 12.4		37.3 ± 14.31		1.948	0.056
BMI (Kg/m^2) Mean ± SD	27.2 ± 3.67		28.2 ±1.7		-0.862	0.395
Nail changes, N (%)	23 (76.7%)		-		-	-
Dactylitis, N (%)	5 (16.7%)		-		-	-
Enthesopathy, N (%)	19 (63.3%)		-		-	-
Sacroiliitis, N (%)	15 (50%)		-		-	-
	Median (IQR)	**Range**	**Median (IQR)**	**Range**		
Number of tender joints	26.5 (6.75 –43.25)	1-63	-	-	-	-
Number of swollen joints	1.5 (0 – 2.25)	0-10	-	-	-	-
PASI	10.05 (1.2 – 22.3)	0.3 – 66.6	-	-	-	-
DAPSA	48 (24.15–68.25)	3.6 – 96.15	-	-	-	-
ESR (mm/hr)	16.5 (8– 30)	4–61	9 (8–10)	3–20	-	0.021*
CRP (mg/L)	4 (1.13 – 8.05)	0.6–18	2 (1.2–5)	0.4–8	-	0.004*
Serum Orsomucoid (ng/mL)	38.53 (21.57– 74.6)	13.28– 115.6	22.35 (17.4-29.42)	11.4– 72.5	-	0.007*
Urine Orsomucoid (ng/mL)	0.6 (0.42 – 0.68)	0.262 – 0.952	0.262 (0.22– 0.30)	0.175– 0.532	-	<0.001**
OrsU/creatinineU ratio (ng/mL)	0.01 (0.008– 0.125)	0.06– 0.16	0.005 (0.024– 0.006)	0.002– 0.009	-	<0.001**

χ2: Chi square test.
t: independent sample t-test.
** P<0.05 was considered statistically significant.*
*** P ≤0.001 was considered statistically highly significant.*
BMI, Body mass index; CRP, C reactive protein; ESR, DAPSA, Disease Activity index for Psoriatic Arthritis; Erythrocyte sedimentation rate; OrsU/creatinine U ratio, orsomucoid in urine/ creatinine in urine ratio; PASI, Psoriasis Area and Severity Index; PsA, Psoriatic arthritis.

Table (2): Relation between characteristics of psoriatic arthritis patients and disease activity

	Remission-to-low (n=7)	Moderate-to-high (n=23)	χ^2	p
	N (%)	N (%)		
Dactylitis Present	0 (0%)	5 (21.7%)	F	0.304
Enthesopathy Present	0 (0%)	19 (82.6%)	F	<0.001**
Sacroiliitis Positive	0 (0%)	15 (65.2%)	F	0.006*
	Mean ± SD	**Mean ± SD**	**t**	**p**
Age	44.29 ± 10.87	43.96 ± 13.06	0.06	0.952
BMI	26.29 ± 4.88	27.5 ± 3.3	-0.761	0.453
	Median (IQR)	**Median (IQR)**	**Z**	**p**
Disease duration	8 (5 – 18)	15 (7 – 20)	-1.455	0.146
DAPSA score	10.84 (5.17 – 15)	57 (45 – 73)	-3.948	<0.001**
ESR	8 (5 – 12)	24 (9 – 40)	-2.727	0.006*
CRP	1 (0.8 – 1.17)	6 (2.26 – 9.01)	-3.242	0.001**
Treatment				
Biological	2 (28.6%)	6 (26.1%)		
DMARDs	1 (14.3%)	8 (34.8%)		
No	3 (42.9%)	7 (30.4%)	MC	0.9
Non-specific	1 (14.3%)	2 (8.7%)		

χ2: Chi square test.
F: Fisher's exact test.
t: independent sample t-test.
Z: Mann-Whitney test
** P<0.05 was considered statistically significant.*
*** P ≤0.001 was considered statistically highly significant.*
BMI, Body mass index; CRP, C reactive protein; ESR, DAPSA, Disease Activity index for Psoriatic Arthritis; DMARDs, Disease modifying anti-rheumatic drugs; Erythrocyte sedimentation rate.

Table (3): Correlation between serum, urine Orsomucoid and laboratory parameters

	Serum Orsomucoid		Urine Orsomucoid	
	r	p	r	p
Age (year)	-0.338	0.057	-0.108	0.571
BMI (kg/m^2)	-0.092	0.682	-0.195	0.301
Disease duration (year)	-0.162	0.391	-0.13	0.495
Number of tender joints	0.093	0.624	0.711	<0.001*
Number of swollen joints	-0.17	0.369	-0.273	0.144
PASI score	-0.041	0.831	-0.233	0.216
DAPSA	0.166	0.379	0.741	<0.001*
ESR (mm/hr)	0.161	0.395	-0.281	0.133
CRP (mg/L)	0.009	0.961	-0.346	0.061

r: Spearman rank cocorrelation coefficient.
** P ≤0.001 was considered statistically highly significant.*
BMI, Body mass index; CRP, C reactive protein; ESR, DAPSA, Disease Activity index for Psoriatic Arthritis; PASI, Psoriasis area and severity index).

Table (4): Performance of Orsomucoid in the diagnosis of psoriatic arthritis

	Cutoff	AUC	Sensitivity	Specificity	PPV	NPV	Accuracy	p
Serum ORM	≥25.031	0.704	70%	66.7%	67.7%	69%	68.3%	0.007*
Urinary ORM	≥0.306	0.936	86.7%	86.7%	86.7%	86.7%	86.7%	<0.001**

** P<0.05 was considered statistically significant.*

*** P ≤0.001 was considered statistically highly significant.*

AUC, Area under the curve; NPV, Negative predictive value; ORM, Orsomucoid; PPV, Positive predictive value.

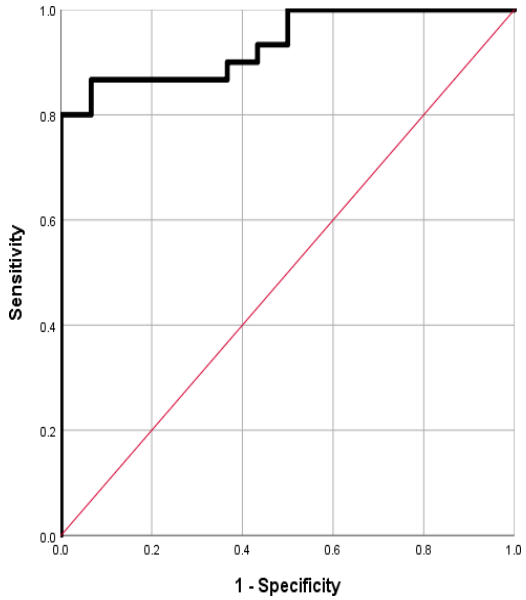

Figure (1): ROC curve showing performance of urine Orsomucoid in the diagnosis of psoriatic arthritis.

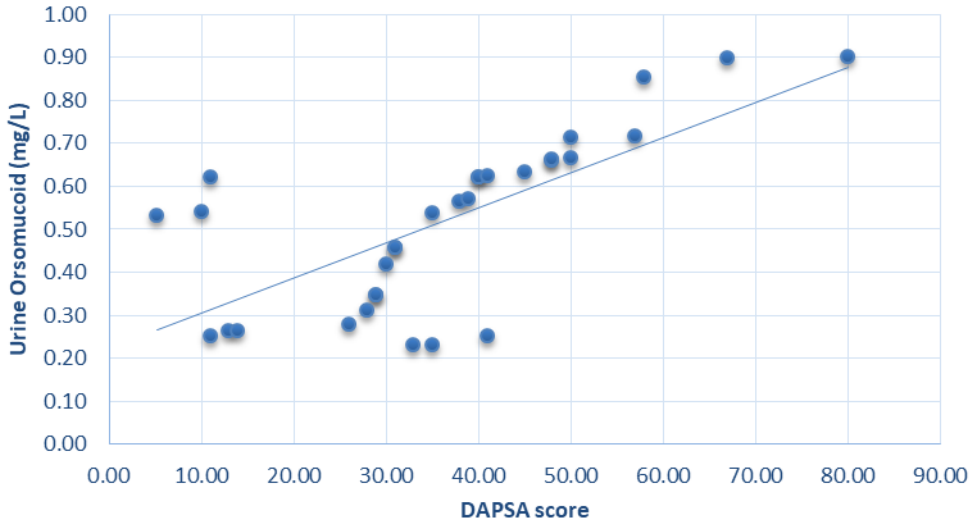

Figure (2): Scatter dot plot showing significant positive correlation between urine Orsomucoid and DAPSA score (*r=0.742, P <0.001*).

DISCUSSION

One type of persistent inflammatory arthritis is psoriatic arthritis. It affects about 20% of people who have psoriasis. This severe illness is marked by a lowered quality of life and possible serious morbidity [8]. An acute phase protein (APP) that is mostly produced in the liver is called orsomucoid (ORM). This protein was once utilized in the diagnosis and follow-up of autoimmune diseases like rheumatoid arthritis, systemic lupus erythematous, inflammatory bowel disease, multiple sclerosis, and adult-onset Still's disease that are linked to persistent inflammatory activation. Additionally, it increases the incidence of diabetes mellitus, cancer, and infections [4, 9]. For the best possible care and early detection of consequences, disease activity must be monitored. This is the first study to look at orsomucoid protein A levels in the urine and serum of Egyptian individuals with psoriatic arthritis (PsA). We discovered that PsA patients had considerably higher ESR and CRP levels than the control group. This was in line with the findings of Yurdakul et al., who reported that in psoriatic individuals, inflammatory indicators are linked to arthritis [10]. We discovered that PsA patients had considerably greater serum and urine orsomucoid levels than healthy persons, which is consistent with earlier research. When compared to healthy people, Maranini et al. discovered that patients with adult-onset Still's disease had higher expression levels of the orsomucoid 1 protein [11]. These results corroborated those of Biljan et al., who found that psoriatic patients had considerably greater serum ORM than the control group [9] in relation to orsomucoid. These results disagreed with those of Khalid et al. [12] who found no statistically significant difference between the control group and psoriatic patients. We discovered that PsA patients had a noticeably increased orsomucoid/creatinine urine ratio in contrast to people in good health. These results were consistent with those of Kustán et al. [13] who found that psoriatic patients had considerably greater u-ORM and u-ORM/u-CREAT ratios than controls. A statistically significant correlation was seen between the occurrence of enthesopathy and disease activity. These results corroborated those of Palominos et al. [14], who discovered that enthesitis is linked to increased disease activity in individuals with psoriatic arthritis. Because the majority of our patients exhibited higher disease activity, Ahmad et al. [15] discovery of a slight connection between soreness and inflammatory or damage scores at the tendo-Achilles enthuses may explain this finding.

We found no statistically significant relation between disease activity and dactylitis. Kaeley et al. [16] found that dactylitis is associated with more erosive forms of PsA, and this could be attributed to the small number of patients having dactylitis in our study (five patients). We found no correlation between urine ORM and CRP. These results conflicted with those of El-Beblawy et al. [17], who discovered an independent relationship between hs-CRP and serum and urine ORM in individuals with Type 1 Diabetes. This finding may be explained by the fact that hs-CRP is more sensitive than CRP. Although other studies showed an association between ORM and PASI score in psoriatic patients [12].We found no correlation between ORM and PASI score. We found no correlation between ORM and ESR or CRP. Our findings were in disagreement with Park et al. [18], who reported that ORM showed a weak correlation with ESR and CRP in psoriasis patients. We found no relation between skin severity and either serum or urine ORM. Serum ORM levels were found to be consistent with Khalid et al.'s findings [12], which indicated that there was no meaningful correlation between the disease's severity and serum ORM level. Urinary ORM findings contradicted those of Khalid et al. [12], who reported a significantly significant relationship between the severity of the disease and the u-ORM level. A noteworthy correlation was seen between disease activity and urine ORM. These results were consistent with previous research that demonstrated elevated u-ORM excretion in urine, which is thought to be connected to immune system activation [19]. Urinary ORM levels in patients with rheumatoid arthritis had a positive association with the disease activity state, as demonstrated by Park et al. [18]. Vilela et al. [20] proved that ORM correlates with the disease activity of inflammatory bowel disease.

CONCLUSION

In patients with PsA, serum and urine levels of orsomucoid are considerably higher. Orsomucoid cysts in the urine are linked to active illness. Orsomucoid may be a useful biomarker for psoriatic arthritis patients to assess the disease's activity and may play a part in the pathophysiology of the condition.

Conflict of interest: None.

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