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ORIGINAL ARTICLE

Assessment of Serum Osteopontin Level in Psoriatic Patients Associated with Hepatitis C Virus Infection

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ABSTRACT

Background: *Hepatitis C virus (HCV) is associated with many dermatoses including psoriasis through stimulating inflammatory cells to infiltrate skin lesions.* ***Objectives****: To assess serum osteopontin (OPN) in psoriatic patients associated with HCV infection and its relation to severity and progression of psoriasis.* ***Methodology:*** *OPN was assayed by ELISA for 60 patients with psoriasis and 30 healthy individuals as control group.* ***Results:*** *OPN levels (pg/ml) were 49.8±30.2; 110.9±50.2 and 284.0±45.3 in mild, moderate and severe psoriasis; respectively. OPN levels (pg/ml) were increased in 21(35%) psoriatic patients with positive HCV antibodies (182.1±47.4) as compared to negative patients (49.87±13.5). OPN correlated positively (r= 0.81; p< 0.0001) with psoriasis lesions and severity of psoriasis calculated by psoriasis area and severity index(PASI) score. Area under the ROC of OPN to discriminate psoriasis patients from healthy individuals; mild severity from moderate severity; mild severity from severe; moderate severity from severe were 0.93;0.81; 0.88 and 0.86; respectively. HCV prevalence were 10.8%, 42.9% and 72.8% in mild, moderate and severe; respectively.* ***Conclusion:*** *There is a relation between serum OPN and HCV infection in psoriatic patients and level of OPN correlate with severity of psoriasis and also reflects progression of psoriasis in HCV infected patients.*

INTRODUCTION

Psoriasis is a common chronic inflammatory disease of the skin and joints, affecting 2–4% of the population[1]. Psoriasis is characterized by an exaggerated proliferation of keratinocytes (KCs) secondary to an activated immune system[2]. Egypt has the highest prevalence of HCV infection in the world, affecting an average of 15–25% of the population in rural communities[3]. Many skin disorders, including psoriasis, have been described in association with HCV. There is suggestion that HCV infection may act as trigger for systemic inflammation independent from the persistence of active infection. Increased psoriasis area and severity index (PASI) in HCV positive patients could be explained by common pathogenic mechanisms in psoriasis and HCV infection including production of interferon[4]. OPN is a multifunctional glycophosphate protein which during inflammation is expressed by natural killer cells, activated T cells, osteoblasts, vascular smooth muscle cells and macrophages[5]. OPN levels in psoriatic patients were significantly elevated in comparison to healthy controls suggesting a possible role of OPN in the development of psoriasis. Elevated OPN was associated with upregulation of IL–1, TNF and IFN–gamma, whereas IL–10 and IL–4 were down-regulated. These results suggest the possible role of OPN as an early cytokine able to drive Th–1 response in psoriatic patients[6]. To the best of our knowledge, the relation between OPN and severity of psoraisis in HCV infected patients was not assessed. The aim of our study was to assess the serum level of OPN in psoriatic patients associated with HCV infection and to investigate whether serum OPN reflect extensive lesions and progression of psoriasis.

METHODOLOGY

This was a case control study and included 60 psoriatic patients who had attended to Dermatology Outpatient Clinic at Damietta University hospital, Al-Azhar University. The study also included 30 apparently healthy subjects matched for age and sex as control group. The study was conducted during the period from March 2015 to January 2016. Patients with any other dermatological, cardiovascular, bone or auto-immune diseases were excluded from the study. All individuals

were subjected to full history taking, complete general examinations and dermatological examinations including clinical assessment of psoriasis lesions and calculation of psoriasis area and severity index (PASI) score[7]. Laboratory investigations included assay of liver enzymes (ALT and AST enzymes), HCV-Ab by Immunofluorescence assay (IFA), HCV-RNA by PCR and estimation of serum OPN by ELISA for all participants. All subjects provided informed consent to participate in the study. The study protocol was approved by the Institutional Review Committee and conformed to the ethical guidelines of the 1975 Helsinki Declaration.

Laboratory Tests:

Five ml of venous blood were taken from patients and controls. Blood samples were collected in plain tube, left to clot, centrifuged and serum was separated. liver enzymes were measured on an automated biochemistry analyzer (Beckman Coulter AU480). Serum was used to determine anti-HCV antibodies by Immunofluorescence assay (IFA) on Minividas autoanalyzer. Quantification of HCV-RNA was done in positive cases using Applied Biosystem Real Time PCR (Model 7500 system, Singapore).

Measurement of serum OPN Level:

OPN level was determined using human OPN ELISA kit (Catalog No.CSB-E08392h, CUSBIO, China). Diluted serum sample (1:10) with the assay diluent and standard (100 ul) were pipetted into the wells (100 µl), then incubated for two hours at 37°C with gentle shaking. After four washes, 100 µl of biotinylated second antibody was added and incubated for one hour at room temperature. with gentle shaking. After four washes, 100µl of diluted HRP conjugated-streptavidin was added and incubated for 60 minutes at room temperature with shaking. The ELISA microplate was again washed for 5 times, then 100µl of tetramethyl-benzidine (TMB) substrate was added and incubated exactly for 30 min in the dark with shaking. The reaction was stopped with the stop solution (50µl of 2 M sulfuric acid) before measuring the optical density of each well at 450 nm with a microtiter plate reader (Tecan Austria GmbH, Sunrise-Basic TECAN). The concentration of OPN was determined by interpolation from the standard curve then was multiplied by the dilution factor.

Statistical analysis:

Statistical analyses were performed by SPSS software version 15.0. Continuous variables were expressed as mean ± standard deviation whereas categorical variables were expressed as numbers and percentage. Correlation was evaluated by Spearman's rank correlation coefficients. Statistical differences were tested using ANOVA test, t-test or nonparametric Mann-Whitney test. A value of $P<0.05$ is considered statistically significant. Categorical values were compared using chi-square test. The diagnostic performances of Osteopontin were evaluated using areas under the ROC curves (AUC).

RESULTS

Sixty patients with psoriasis and 30 healthy individuals were included in the study. Psoriatic patients and healthy individuals showed no differences regarding their age and sex but showed significant differences regarding their ALT and AST activity. Regarding incidence of HCV infection in psoriatic patients, it was positive in 21 cases (35%). Regarding HCV-RNA of positive HCV- antibody cases; the mean was of 1127360 ± 37846 IU/ml; table1.

Table 1. Comparison between psoriasis and control groups as regard to clinical and laboratory data

Variables	*Psoriasis*	*Control*	*P value*
Age (years)	41.5±11.4	40.9± 13.5	0.53
Sex			
Male	33 (55%)	15 (50%)	0.45
Female	27 (45%)	15 (50%)	
ALT (U/L)	43.6±6.2	25.9 ±6.31	< 0.001*
AST (U/L)	46.9 ±27.5	38.0±24.9	< 0.001*
HCV-antibody			
Positive	21 (35%)	0 (0%)	< 0.0001*
Negative	39 (69%)	30 (100 %)	
HCV- RNA (IU/ml)	1127360 ± 37846	-	
Osteopontin (pg/ml)	102 ± 23	15.9 ± 4.5	< 0.0001*

P>0.05 is considered not significant; *P< 0.05 considered significant.

Level of Osteopontin:

The mean OPN levels(pg/ml) were significantly elevated in all psoriatic patients (102±23) in comparison to control group (15.9±4.5) (P <0.0001); table 1. It was found that serum OPN levels were not significantly affected by patient sex, duration of illness, family history and psoriasis types. Among psoriatic patients, serum OPN levels were increased with increased severity of disease with highly statistical significant differences (p<0.0001). Its levels were 49.8±30.2;

110.9±50.2 and 284.0±45.3 in mild, moderate and severe cases; respectively. Serum OPN levels (pg/ml) were increased in progressive psoriatic patients (121.3±27.7) than stationary psoriatic patients (51.7±11.7) with significant differences (p= 0.01); table 2; figure 1. Serum OPN levels (pg/ml) were increased in psoriatic patients with positive HCV antibodies (182.1±81.4) as compared to negative patients (49.8±33.5). No significant correlation was found between level of OPN and age, ALT, AST, disease duration, HCV-RNA viral load. Level of OPN correlated positively (r= 0.81; p< 0.0001) with PASI; figure 2. The diagnostic value of OPN to discriminate all patients with psoriasis from healthy individuals was assessed by the area under the ROC (AUC). AUCs of OPN to discriminate all patients with psoriasis from healthy individuals; mild severity from moderate severity; mild severity from severe; moderate severity from severe were 0.93;0.81; 0.88 and 0.86; respectively; figure 3.

Table 2. Level of osteopontin (pg/ml) in psoriasis patients (n= 60)

Variable	*No (%)*	*Osteopontin*	*Value P* [a]
Sex			
Male	33 (55%)	100 ± 87.8	0.69
Female	27 (45%)	113±86	
Duration of illness			0.263
< 5 years	25 (55.6%)	89.6± 65.5	
5 – 10 years	20 (22.2%)	142.3±127.9	
>10 years	15 (22.2%)	96.1±81.6	
Severity			
Mild	28 (46.7 %)	49.8±30.2	
Moderate	21 (35%)	110.9±50.2	< 0.0001
Severe	11 (18.3%)	284.0±45.3	
Course			
Progressive	40 (66.7)	121.3±93.7	0.01
Stationary	20 (33.3)	51.7±25.7	
Family history			
Yes	56 (93%)	26.5±7.8	> 0.05
No	4 (7%)	108.6±86.8	
Psoriasis types			
Vulgaris	40 (66%)	175.1± 78.4	
Pustular	15 (25%)	183.1±69.1	> 0.05
Arthropathy	5 (9 %)	177.1± 59.1	
HCV-Ab			
Positive	21 (35%)	182.1±81.4	< 0.0001
Negative	39 (69%)	49.8±33.5	

[a] P>0.05 is considered not significant; P< 0.05 considered significant.

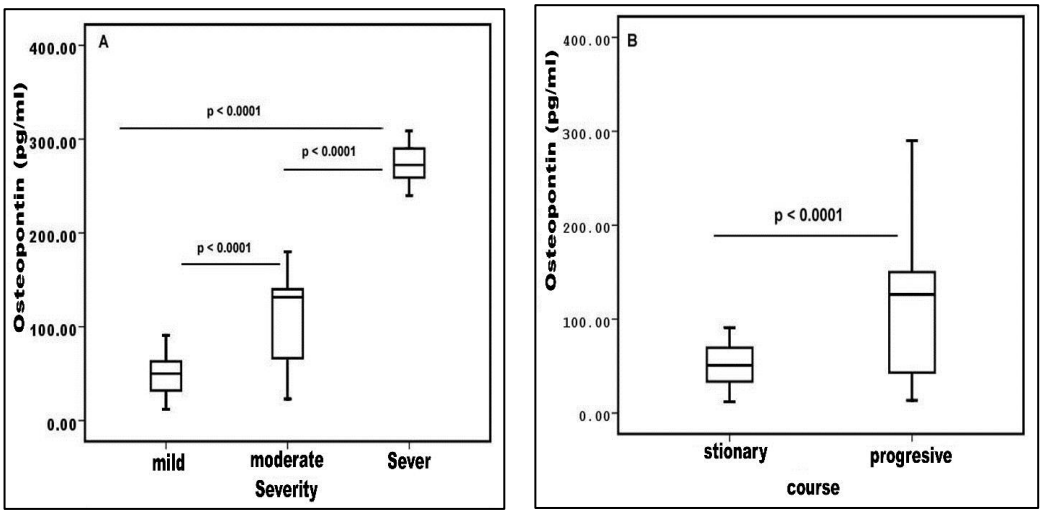

Fig. 1: Box plots for OPN in patients with psoriasis. A. Box plots of OPN in psoriasis severity B. Box plots of OPN in Psoriasis course.

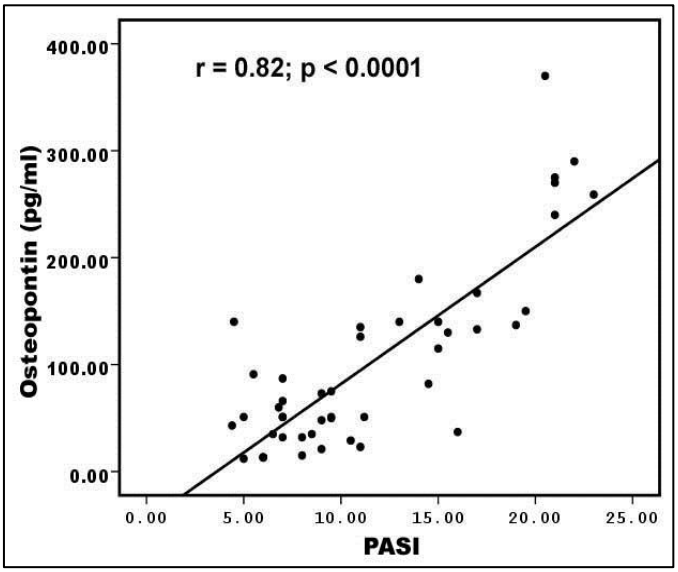

Fig. 2: Correlation between levels of OPN and Psoriasis Area Severity Index (PASI) in all patients.

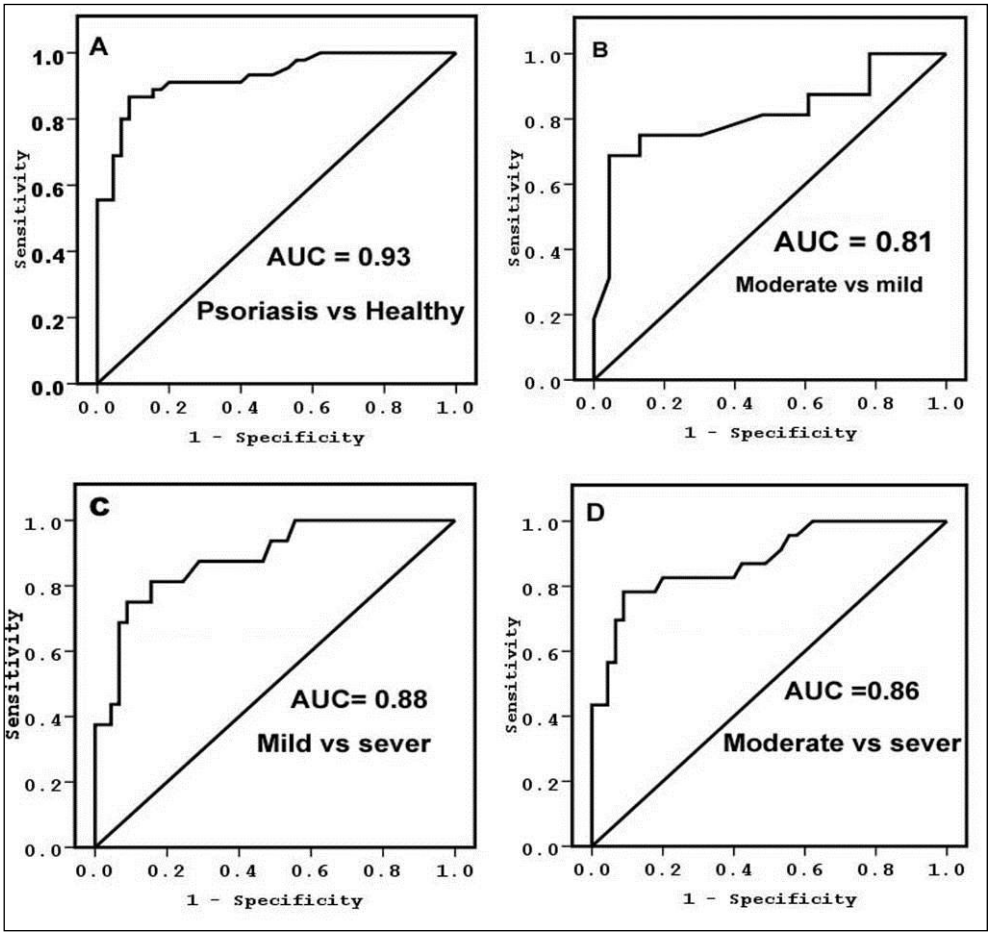

Fig. 3: Diagnostic performances of OPN using Area under ROC curve (AUC). A. AUC of OPN to discriminate all patients with psoriasis from healthy individuals. B. AUC of OPN to discriminate mild severity from moderate severity. C. AUC of OPN to discriminate mild severity from severe. D. AUC of OPN to discriminate moderate severity from severe cases.

Relation between HCV infection and psoriasis clinical features:

The HCV antibody prevalence in psoriatic patients according to various clinical features of psoriatic patients was presented in table 3. It was found that presence of HCV antibody was not significantly affected by patient sex, duration of illness, family history and psoriasis types. Among psoriatic patients, HCV antibody prevalence was increased significantly with increasing severity of psoraisis ($p < 0.0001$). HCV antibody percentage were 10.8%, 42.9% and 72.8% in mild, moderate and severe cases; respectively. Its percentage was increased in progressive psoriatic patients (37.5%) than stationary psoriatic patients (30%) with significant differences ($p= 0.009$); table3.

Table 3. Relation between HCV infection and psoriasis clinical features

Variable	*No (%)*	*HCV-Ab*		*X^2 ; p value*
		Negative	**Positive**	
Sex				
Male	33 (55%)	21(63.6%)	12(36.4%)	
Female	27 (45%)	18 (66.7%)	9 (33.3%)	0.21;0.476
Duration of illness				
< 5 years	25 (55.6 %)	16(64%)	9(63%)	
5 – 10 years	20 (22.2 %)	14(70%)	6(30%)	0.58; 0.74
>10 years	15 (22.2 %)	9(60%)	6(40%)	
Severity				
Mild	28 (46.7 %)	25 (89.2)	3 (10.8%)	17.7: < 0.0001
Moderate	21 (35 %)	12(57.1)	9 (42.9%)	
Sever	11 (18.3 %)	3 (27.2)	9 (72.8%)	
Course				
Progressive	40 (66.7%)	25(62.5%)	15 (37.5%)	
Stationary	20 (33.3%)	14 (70%)	6 (30%)	6.8; 0.009
Family history				
Yes	56 (93%)	35 (62.5%)	21(37.5%)	2.1; 0.343
No	4 (7%)	4(100%)	0 (0%)	
Psoriasis types				
Vulgaris	20 (33.3%)	14 (70%)	6(30%)	
Pustular	20 (33.3%)	12 (60%)	8 (40%)	0.25; 0.41
Arthropathy	20 (33.3%)	13 (65%)	7 (35%)	

[a] P>0.05 is considered not significant; P< 0.05 considered significant.

DISCUSSION

An association between HCV and psoriasis is frequently described in previous studies and other epidemiological survey confirmed a higher prevalence of HCV infection in psoriatic patients[8]**.** Our study found that HCV antibodies were positive in 21 cases (35%) out of 60 psoriatic patients. On the other hand, some studies[9] have shown a relatively low prevalence (8.5%) of HCV infection in psoriatic patients when compared to that of the present study. The possible explanation of higher incidence of HCV infection in psoriatic patient in our study may be due to endemicity of HCV in Egypt. Egypt has the highest HCV prevalence in the world (12% among the general population and reaches 40% in persons 40 years of age and above in rural areas)[10]**.** OPN is an acidic glycoprotein produced by cells of the immune system, epithelial tissue, smooth muscle cells, osteoblasts and tumor cells**.** OPN interacts with integrins and CD44 to enhance Th1 and inhibit Th2 cytokine expression[11].

Plasma levels of OPN have been shown to be higher in psoriasis patients compared with controls, and this elevation is significantly associated with occurrence of psoriasis[12]**.** In the present study, the mean OPN levels were significantly ($P <0.0001$) elevated in all psoriatic patients in comparison to healthy individuals. Among psoriatic patients, serum OPN levels were increased with increased severity of disease with highly significant differences ($p<0.0001$). Also, OPN levels were increased in progressive psoriatic patients than stationary psoriatic patients with significant differences ($p= 0.01$). Several studies investigated the association between the severity of psoriasis and OPN level. High level of OPN in patients with psoriasis can be explained in light of the several studies[13-15]**.** In the present study, level of OPN correlated positively ($r= 0.81$; $p< 0.0001$) with PASI score which suggests that OPN levels reflect psoriasis severity. In this study, serum OPN levels were increased in psoriatic patients with positive HCV antibodies as compared to negative patients suggesting that HCV infection may contribute to the severity of psoriasis. AUCs of OPN to discriminate all patients with psoriasis from healthy individuals; mild severity from moderate severity; mild severity from severe; moderate severity from severe were 0.93;0.81; 0.88 and 0.86; respectively. AUCs of MicroRNAs-223 and 143 were 0.80 and 0.75 for discrimination between

patients with psoriasis and healthy individuals[16]. In the present study, the percent of HCV antibody positivity were 10.8%, 42.9 % and 72.8% in mild, moderate and severe cases respectively which suggests that HCV infection was associated with severity of psoriasis. These results agree with study by Yousef et al. [8] who stated that detection rate of HCV in psoriatic patients was 19% vs 8.5% in controls.

CONCLUSION

Results of our study suggest that there is a relation between serum OPN and HCV infection in psoriatic patients and level of OPN correlate well with severity of psoriasis and reflects progression of psoriasis in HCV infected patients.

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