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Role of osteopontin in psoriasis: An immunohistochemical study

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Abstract
Introduction: Osteopontin (OPN) has been postulated to have a role in several T-helper (Th) 1 and Th 17-mediated diseases including psoriasis (PS), through multiple mechanisms sharing in the onset and worsening of PS, OPN shares in induction of keratinocyte proliferation through inhibiting keratinocyte apoptosis, OPN acts as a proinflammatory agent that participates in the upregulation of Th cell lineages, among which are the Th 1 and Th 17 cells.
Aims and Objectives: The aim of this study was to explore the possible role of OPN in the pathogenesis of PS. Materials and Methods: This case–control study was carried out on 18 patients of chronic plaque PS (mean age 37.61 ± 14.48) and a control group of 18 apparently healthy volunteers (mean age 41.11 ± 11.02 years).
Severity of PS was assessed using the PS area and severity index score. Two skin biopsies were taken from psoriatic patients. The first was taken from the lesional skin and the other from a counter apparently healthy site.
Results: Our results showed statistically significant differences in the expression of OPN, between lesional and nonlesional skin as well as between nonlesional skin and control group ($P \leq 0.001$). In addition, there was a significant difference in the expression of OPN, between control and lesional group.
Conclusions: OPN involvement in PS enlarges the list of cytokines able to stimulate the inflammatory response in this disease, anti-OPN antibodies, may eventually become a useful therapeutic approach in PS.

Keywords: cytokines, osteopontin, psoriasis, psoriasis area and severity index

Introduction
Psoriasis (PS) is a systemic, chronic, relapsing and hyperproliferative immune-mediated skin disorder [1]. It is characterized by inflammatory skin and joint manifestations [2]. PS results from interaction between genetic predisposition and large spectrum of environmental factors that triggers the development of skin lesion. T-helper (Th) 1 and Th 17 mediated inflammation have been proposed to be pathologically essential in PS. [3] Osteopontin (OPN) is a phosphorylated acidic arg-gly-asp containing glycoprotein implicated biologically in bone remodeling; immune system regulation [4].
OPN is adding to the chronic inflammatory status through antiapoptotic effects on effectors T cells. It was found that Langerhans cell (LC), myeloid dendritic cells (mDCs) that express intracellular OPN upon their activation polarize naive T cells toward a Th 17 phenotype. Secreted OPN (sOPN) directly stimulates interleukin (IL)-17 productions by T cells [5].
In PS, OPN has a proangiogenic effect on microvascular endothelial cell. It acts through promoting vessel formation subsequently supporting the influx of inflammatory cells through a mechanism mediated by IL-1 and matrix metalloproteinase-9, induced by OPN and tumor necrosis factor-a, which acts as an angiogenesis promoting factor [6, 7]. One study demonstrated also that OPN expression seems to be related to the CD34 expression, angiogenesis marker, expressed in the endothelial cells of psoriatic lesional skin [8].
The present study was conducted in an attempt to explore the possible role of OPN in the pathogenesis of PS through histopathologic specimens using immunostaining in psoriatic patients as compared to normal skin biopsies in age- and sex-matched healthy controls.

Materials and Methods
This study was conducted in the Dermatology and Venereology Departments. The study included two groups; patients' group included 18 patients of chronic plaque PS. They were 8 males (44.4%) and 10 females (55.6%). Their ages ranged between 12 and 64 years, mean (37.61 ± 14.48). The diagnosis was established by clinical examination. The severity of the disease was assessed using PS area and severity index (PASI) score [9].
Patients had not taken any topical or systemic treatment during the last 3 months. All patients did not show any systemic disease (e.g., coronary heart disease diabetes and psychological diseases) associated with expected increase of OPN level. Eighteen apparently healthy volunteers (10 males and 8 females) served as the second; control group. Their ages ranged from between 12 and 62 years, mean (41.11 ± 11.02).

Methods
Two punch skin biopsies (5 mm diameter each) were taken from psoriatic patients. The first from the lesional skin and the other from a counter apparently healthy site • Skin biopsies obtained from healthy normal controls were done with 5 mm punch biopsy • each specimen of both groups was fixed in formalin and embedded in paraffin to form paraffin blocks. Serial sections were obtained from each block and stained with the following:

1. Stained section was examined under light microscope for histopathological (hematoxylin and eosin [H and E]) characterization of the lesion.
2. Depigmentation of melanin from tissue specimens: Was done according to method described earlier [10] as follows: Paraffin-embedded specimens were subjected to bleaching

employing two methods. The first employed potassium permanganate with oxalic acid, and the second 10% hydrogen peroxide.

3. To determine optimal bleaching conditions, depigmentation was carried out at various incubation times. The effect of diluents used for 10% H2 O2 was assessed using phosphate-buffered saline and deionized water. All tissues were stained in H and E for histological evaluation.
4. Immunohistochemical staining using monoclonal antibodies:

Immunohistochemical procedure according to Bancroft and Gamble [11]. It was graded as follows: [12] No infiltrate, mild infiltrate, moderate infiltrate, and severe infiltrate. The degree of immunostaining for OPN was evaluated according to the level of epidermal and dermal staining. In the epidermis, three groups were distinguished, basal layer only, lower half of the epidermis, and the whole epidermis [8].

In the dermis, four groups were distinguished: Negative, weak positive, moderately positive, and strongly positive [13]. Data were checked, entered, and analyzed using EPI-INFO 6 for data processing and statistics. $P < 0.05$ indicated significant results.

Results

This study included 18 chronic plaque PS patients as well as 18 control subjects whose demographic and clinical data are presented in Tables 1and 2.

Table 1: Demographic data of patients with psoriasis

Variable	*n*=18
Age	
Range	12-64
Mean±SD	37.61±14.48
Sex, *n* (%)	
Male	8 (44.4)
Female	10 (55.6)
Duration of the disease in month	
Range (m)	2-360
Mean±SD	70.78±89.39
Family history, *n* (%)	
−ve	9 (50)
+ve	9 (50)
Associated disease, *n* (%)	
No	12 (66.7)
HTN	1 (5.6)
CVSD	3 (16.6)
Both	2 (11.1)
Distribution, *n* (%)	
UL	3 (16.86)
Trunk	1 (5.6)
UL + trunk	2 (11.1)
UL + LL	3 (16.6)
H + LL	1 (5.6)
LL + trunk	1 (5.6)
UL + LL + trunk	4 (22.2)
UL + LL + H	1 (5.6)
UL + LL + H + trunk	2 (11.1)

SD: Standard deviation, UL: Upper limb, LL: Lower limb, H: Head, HTN: Hypertension, CVSD: Cardiovascular disorder, +ve: Positive, −ve: Negative

There were statistically highly significant differences in the density of dermal inflammatory infiltrate in psoriatic patients versus controls [Table 3]. A statistically highly significant difference of OPN expression in the dermal inflammatory infiltrate of lesional and nonlesional skin of psoriatic patients versus control as regards density of dermal inflammatory infiltrate was found. Another, a statistically highly significant difference was reported in OPN expression in layers of the epidermis of lesional and nonlesional skin of psoriatic patients versus controls [Table 4].

Moreover, there was a statistically highly significant difference between OPN expression in the layers of the epidermis and OPN expression in dermal inflammatory infiltrate in lesional skin of psoriatic patients [Table 5]. There was a highly statistically significant difference between OPN expression in the epidermis and density of dermal inflammatory infiltrate in psoriatic patients [Table 6].

There was a highly statistically significant difference between OPN expression in the dermal inflammatory infiltrate, and density of dermal inflammatory infiltrate in psoriatic patients [Table 7].

Table 2: Demographic data of the control group

Variable	*n*=18
Age	
Range	21-62
Mean±SD	41.11±11.02
Sex, *n* (%)	
Male	10 (55.6)
Female	8 (44.4)
Past history, *n* (%)	
+ve	0 (0)
−ve	18 (100)
Family history, *n* (%)	
+ve	0 (0)
−ve	18 (100 nb)

SD: Standard deviation, +ve: Positive history of psoriasis or cardiovascular diseases, −ve: Negative history of psoriasis or cardiovascular diseases

Table 3: Psoriatic patients versus controls as regards density of dermal inflammatory infiltrate in skin biopsy

Density of infiltration	Control *n* (%)	Patients with psoriasis *n* (%)		χ^2	*P*
		Nonlesional skin	Lesional skin		
No	18 (100)	7 (38.9)	0 (0)	65.16	0.000*
Mild	0 (0)	8 (44.4)	0 (0)		
Moderate	0 (0)	3 (16.7)	7 (38.9)		
Severe	0 (0)	0 (0)	11 (61.1)		
A=Control versus nonlesional				15.84	0.000*
B=Lesional versus nonlesional				27.60	0.000*
C=Control versus lesional				36.00	0.000*

*Highly significant

Table 4: Osteopontin expression in layers of the epidermis in psoriatic patients versus controls

Layer of the epidermis	Control *n* (%)	Case *n* (%)		χ^2	*P*
		Nonlesional skin	Lesional skin		
Basal layer	18 (100)	7 (38.9)	0 (0)	57.14	0.000*
Lower half of the epidermis	0 (0)	11 (61.1)	5 (27.8)		
The whole epidermis	0 (0)	0 (0)	13 (72.2)		
A=Control versus nonlesional				15.4	0.000*
B=Lesional versus nonlesional				22.25	0.000*
C=Control versus lesional				36.00	0.000*

*Highly significant

Table 5: Osteopontin expression in the epidermis versus osteopontin expression in the dermal inflammatory infiltrate in lesional skin of psoriatic patients

OPN in dermis	OPN expression in the epidermis *n* (%)		χ^2	*P*
	Lower 1/2	Whole epidermis		
Moderate	3 (60)	0 (0)	9.36	0.002*
Marked	2 (40)	13 (100)		

*Highly significant. OPN: Osteopontin

Table 6: Relation between osteopontin expression in the epidermis and density of dermal inflammatory infiltrate in psoriatic patients

Density of infiltration	OPN expression in the epidermis *n* (%)		χ^2	*P*
	Lower 1/2	Whole epidermis		
No	0 (0)	0 (0)	10.88	0.001*
Mild	0 (0)	0 (0)		
Moderate	5 (100)	2 (15.4)		
Severe	0 (0)	11 (84.6)		

*Highly significant. OPN: Osteopontin

There was no statistically significant difference in OPN expression in layers of the epidermis of lesional skin as regards sex of psoriatic patients, but there was a highly significant statistical difference between OPN expression in layers of the epidermis as regards age and duration of disease among psoriatic patients.

There was a statistically insignificant difference in OPN expression in the dermal inflammatory infiltrate of lesional skin as regards the sex and age of psoriatic patients but a significant statistical difference was found in OPN expression in the dermal inflammatory infiltrate as regards the duration of disease among psoriatic patients.

There was a highly statistically significant difference between OPN expression in the layers of the epidermis of lesional skin of psoriatic patients and PASI score as with PASI score mean (6.04 ± 2.99), OPN expression was in the lower 1/2 of the epidermis while with PASI score mean (16.01 ± 4.02), OPN expression was in the whole epidermis.

Table 7: Relation between osteopontin expression in the dermal inflammatory infiltrate and density of dermal inflammatory infiltrate in psoriatic patients

Density of infiltration	OPN expression in the dermis *n* (%)		χ^2	*P*
	Moderate	Marked		
No	0 (0)	0 (0)	5.66	0.017*
Mild	0 (0)	0 (0)		
Moderate	3 (100)	4 (26.7)		
Severe	0 (0)	11 (73.3)		

*Highly significant. OPN: Osteopontin

Discussion

PS is a chronic inflammatory immune-mediated disease. PS is not just a disease of skin and joints, but also is a systemic disease that is connected with a range of co-morbidities, especially metabolic syndrome and cardiovascular disease [2]. This dermatosis is considered to be a T-cell-mediated disease with active hyperproliferation of keratinocytes and abnormal vascular expansion within the superficial dermis.

This expansion is mediated by angiogenesis, an active vasoproliferative process which appears to be a key inflammatory response early in the pathogenesis of PS [14].

OPN has been recently recognized as a potential inflammatory cytokine having a role in many diseases. sOPN appears to have a role in the development of PS through inhibiting keratinocyte apoptosis, thereby supporting enhanced epidermal proliferation; sOPN promotes vessel formation subsequently supporting the influx of inflammatory cells. OPN has a proangiogenic effect on microvascular endothelial cells and has been involved in the onset of angiogenesis through a mechanism mediated by IL-1 [5].

The present study was designed to detect OPN histopathologically in skin specimens of psoriatic patients compared with controls. In our study, there was a statistically significant difference between psoriatic patients and controls regarding density of dermal inflammatory infiltrate.

In controls, there was no dermal inflammatory infiltrate at all while mild to moderate infiltrate was found in nonlesional skin and moderate to severe infiltrate was found in lesional skin of psoriatic patients. While Murphy *et al*. [15] had found neutrophils in psoriatic skin lesions within the epidermis associated with spongiosis (spongiform pustules), neutrophils beneath the cornified layer (subcorneal pustules), neutrophils within the cornified and parakeratotic horn, hypogranulosis, and more keratinocytic mitotic figures above the basal cell layer.

A statistically significant difference was found between psoriatic patients and controls regarding OPN expression in the epidermal skin layers between lesional and nonlesional skin, nonlesional and control group, and finally, lesional skin and control group. OPN was positive in (61.1%) of nonlesional skin at the lower half of the epidermis than its level in lesional skin.

In addition to that, OPN was statistically significantly expressed throughout the epidermis of psoriatic lesion (P = 0.000), while was expressed only in the basal cell layer of normal skin. These findings were in accordance with El-Eishi *et al*. [16] and Amin and Azim [8] who had found that lesional

skin of psoriatic patients showed a statistically significant elevation of OPN levels in comparison to controls.

Amin and Azim [8] had observed that there was a statistically significant difference in OPN expression between lesional and nonlesional skin, nonlesional and control group, and finally, lesional skin and control group, OPN in normal skin was limited to the basal cell layer, hair follicles, sebaceous glands, and sweat glands. In addition to that, in lesional psoriatic skin; OPN positive reactivity was observed and distributed throughout the epidermis at various levels. In nonlesional skin, OPN expression was detected in the epidermis of 71.4% of cases and at lower level.

Similarly, Buommino *et al*. [17] had reported that the level of OPN in both lesional and nonlesional skin of psoriatic patients was significantly higher than the normal skin of controls. This cytokine was found in three-quarter of lesional skin samples, and in 25% of nonlesional skin specimens. These results possibly indicating that in some psoriatic patient's, nonlesional skin might be predisposed to develop the disease, as it can occur in the so-called Koebner phenomenon may be due to elevated OPN expression.

These results were also in agreement with Wrone-Smith *et al*. [18] who stated that, in normal human skin, keratinocytes in the superficial layer of the epidermis undergo apoptosis and there was proliferation of cells in the basal layer. As opposed to normal skin, keratinocytes derived from psoriatic plaques were shown to be resistant to apoptosis. Moreover, inappropriate regulation of apoptosis was proposed as a possible explanation for epidermal thickening in hyperproliferative inflammatory skin disorders including PS [19].

Meanwhile, other study reported statistically insignificant difference in tissue OPN expression between lesional and nonlesional skin of psoriatic patients. Moreover, tissue OPN did not correlate with plasma OPN (P = 0.213) while significant differences as regards tissue OPN between lesional skin of psoriatic patients and normal skin of controls; nonlesional skin of psoriatic patients and normal skin of controls were reported [20].

Similarly, Abdou *et al*. [21] had found that OPN was expressed in the epidermis of all specimens, both in the PS group and the control group without any significant differences except for the tendency of psoriatic lesions to show more cytoplasmic and nuclear pattern of OPN staining (55.56%) compared to normal skin (20%). In our study, OPN was expressed in the dermal inflammatory cells of lesional and nonlesional skin of psoriatic patient and there were statistically significant differences between psoriatic patients and controls as regards OPN expression in the dermal inflammatory infiltrate in lesional, nonlesional, and controls skin.

OPN was not expressed at all in the dermal inflammatory infiltrate in controls but in lesional skin was moderately expressed in 7 patients and strongly expressed in 11 patients. In nonlesional skin of patients, OPN showed no expression in 7 patients, mild expression in 8 patients and moderate expression in 3 patients. In addition to that, there was a statistically significant difference between OPN expression in the layers of the epidermis and OPN expression in dermal inflammatory infiltrate in psoriatic patient lesional skin.

In agreement with our study, Amin and Azim, [8] had found that in lesional skin, OPN was expressed in the inflammatory cells and microvasculature endothelial cells of the dermis in all cases, while in nonlesional skin, positive staining of less intensity was observed in lymphocytes and microvasculature endothelial cells of the dermis in 35.7% of patients.

Conclusion

Our study suggests that OPN involvement in PS enlarges the list of cytokines able to stimulate the inflammatory response in this disease. OPN is involved in the pathophysiology of PS, in the onset and worsening of PS and finally, a possible association with disease severity, OPN acts by different mechanisms through its expression by lesional keratinocytes, inflammatory cells, and endothelial cells.

References

1. Mahajan R, Handa S. Pathophysiology of psoriasis. Indian J Dermatol Venereol Leprol. 2013; 79(7):S1-9.
2. Kilic B, Dogan U, Parlak AH, Goksugur N, Polat M, Serin D *et al*. Ocular findings in patients with psoriasis. Int J Dermatol. 2013; 52:554-9.
3. Abdelnoor AM, Al-Akl N. Factors involved in the pathogenesis of psoriasis. Adv Stud Med Sci. 2013; 1:75-94.
4. Bassyouni IH, Bassyouni RH, Ibrahim NH, Soliman AF. Elevated serum osteopontin levels in chronic hepatitis C virus infection: Association with autoimmune rheumatologic manifestations. J Clin Immunol. 2012; 32:1262-9.
5. Buback F, Renkl AC, Schulz G, Weiss JM. Osteopontin and the skin: Multiple emerging roles in cutaneous biology and pathology. Exp Dermatol. 2009; 18:750-9.
6. Nissinen L, Kähäri VM. Matrix metalloproteinases in inflammation. J Biochim Biophys Acta. 2014; 1840:2571-80.
7. Delort L, Jardé T, Dubois V, Vasson MP, Caldefie-Chézet F. New insights into anticarcinogenic properties of adiponectin: A potential therapeutic approach in breast cancer? Vitam Horm. 2012; 90:397-417.
8. Amin MM, Azim ZA. Immunohistochemical study of osteopontin, Ki-67, and CD34 of psoriasis in Mansoura, Egypt. Indian J Pathol Microbiol. 2012; 55:56-60.
9. Matteo C, Michela C. Psoriasis Area Severity Index (PASI) Calculator 2013. (1.7.1). Available from: http://www.pasi. corti.li. [Last accessed on 2014 Feb 13].
10. Manicam C, Pitz S, Brochhausen C, Grus FH, Pfeiffer N, Gericke A. Effective melanin depigmentation of human and murine ocular tissues: An improved method for paraffin and frozen sections. PLoS One. 2014; 9:e102512.
11. Bancroft JD, Gamble M. Theory and Practice of Histological Techniques. 5th ed. Edinburgh: Churchill Livingstone. 2002, 796.
12. Erdogan D, Kloek JJ, ten Kate FJ, Rauws EA, Busch OR, Gouma DJ *et al*. Immunoglobulin G4-related sclerosing cholangitis in patients resected for presumed malignant bile duct strictures. Br J Surg. 2008; 95:727-34.
13. Shi SR, Gu J, Kbalar KL, Chen T, Cote RJ, Taylor CR. Antigen retrival technique: A novel approach to immunohistochemistry on routinely processed tissue sections. In Analytical Morphology book, Gu J. 1st ed. New York: Springer Science, Business Media. 1997, 1-40.
14. Dogan S, Atakan N. Psoriasis: A disease of systemic inflammation with comorbidities. In: Lima H, editor. PsoriasisTypes, Causes and Medication. 1st ed. Rijeka, Croatia: In Tech Europe. 2013, 107-80.

15. Murphy M, Kerr P, Grant-Kels JM. The histopathologic spectrum of psoriasis. Clin Dermatol. 2007; 25:524-8.
16. El-Eishi NH, Kadry D, Hegazy RA, Rashed L. Estimation of tissue osteopontin levels before and after different traditional therapeutic modalities in psoriatic patients. J Eur Acad Dermatol Venereol. 2013; 27:351-5.
17. Buommino E, Tufano MA, Balato N, Canozo N, Donnarumma M, Gallo L *et al*. Osteopontin: A new emerging role in psoriasis. Arch Dermatol Res. 2009; 301:397-404.
18. Wrone-Smith T, Mitra RS, Thompson CB, Jasty R, Castle VP, Nickoloff BJ. Keratinocytes derived from psoriatic plaques are resistant to apoptosis compared with normal skin. Am J Pathol. 1997; 151:1321-9.
19. Koçak M, Bozdogan O, Erkek E, Atasoy P, Birol A. Examination of Bcl-2, Bcl-X and bax protein expression in psoriasis. Int J Dermatol. 2003; 42:789-93.