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RESEARCH ARTICLE

UTILITY OF VEGF, Ki67, AND GLUT-1 IMMUNOHISTOCHEMICAL MARKERS IN DIFFERENTIATING PALMOPLANTAR PSORIASIS FROM CHRONIC ECZEMATOUS LESIONS

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ABSTRACT

Background: Based on clinical and histomorphological study, differentiating Chronic Plaque Psoriasis from chronic eczematous lesions of palmoplantar region is a diagnostic challenge, however, it has some implications for the patient's management and prognosis.
Aim: This study is designed to evaluate immunohistochemical markers for distinguishing psoriasis from eczema of palmoplantar region in patients referring to Razi Skin Hospital, Tehran, Iran.
Materials and Methods: Comparative cross-sectional study was carried out at Razi Skin Hospital, Tehran, Iran. Immunohistochemical stains for VEGF, Ki67, and GLUT-1 were performed on 45 cases including 15 patients with palmoplantar psoriasis, 15 patients with palmoplantar eczematous lesions, and 15 patients with diagnosis of lichen planus as control group.
Results: The expression of Ki-67 were not significantly different between psoriatic and eczematic lesions but it was more severe in patients with psoriasis. VEGF staining showed a higher vascular proliferation in psoriasis than in eczema and lichen planus. There was no significant difference between GLUT1 expression in 3 examined groups but most cases of psoriasis show diffuse distribution for GLUT1 staining compared with eczema and lichen planus, which mostly show discontinuous staining for GLUT1 marker.
Conclusion: Our findings indicate that VEGF immunostaining shows a significantly more expressed vascular proliferation in patient with psoriatic lesions, and can be helpful as immune marker for differentiating palmoplantar psoriasis and eczema of this region.

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INTRODUCTION

Psoriasis is a common chronic autoimmune disease that affects 2% of population worldwide which impairs patient's life style and causes physical and psychological burdun. (Kurd *et al*., 2007) Plaque-type psoriasis the most frequent form and accounts for 85%–90% of patients with psoriasis. This type of psoriasis is characterized by raised inflamed skin lesions with silvery-white scaly coverings scalp, elbows, knees, hair and nails are typical sites of involvement. Palms of the hands and sole regions can also be involved which can be accompanied with psoriasis in typical regions such as elbows, knees, hair and nails. (Langley *et al*., 2005) Differentianting palmoplantar psoriasis from eczematous dermatitis at these sites is a diagnostic challenge especially in the cases without involvement of typical regions. (Abdou *et al*., 2013) Palmoplantar psoriasis and eczematous dermatitis share common clinical and histomorphologic features, therefore, utilization of immunohistochemical study is under debate. (Kamyab Hesari *et al*., 2014) The present study was designed to evaluate immunohistochemical markers for VEGF, Ki67, and GLUT-1 in distinguishing palmoplantar psoriasis from eczema. The mentioned marker were chosen by considering pathogenesis of psoriasis. The pathogenesis of psoriasis involves abnormal epidermal differentiation hyperproliferation, and angiogenesis as well as markedly shortened turnover time. (Abdou *et al*., 2013; van Erp *et al*., 1989; Leigh *et al*., 1985) T-

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cell mediated immunity is also the accepted mechanism in pathogenesis of psoriasis. Many inflammatory cytokines, growth factors, angiogentic factors are known to be increased in this process. (Boehncke, 2015) Ki-67 is regarded as a well-established marker for evaluation of cellular proliferative activity. Higher expression of Ki67 in psoriatic lesions is correlated with severity of the disease. (Heng *et al*., 2000) Glucose transporter 1 (GLUT-1) facilitates transport of glucose in mammalian cells which was required for rapid proliferation in cancer cells and over expressed in cases of benign and malignant neoplasms as well as inflammatory conditions such as psoriasis. (Abdou *et al*., 2013; Tao *et al*., 2008) VEGF is a pro-angiogenic factor which shows over expression in psoriatic lesions and postulated to have a rule in pathogenesis of this lesions. (Marina *et al*., 2015) Assessment of the mentioned factors can provide us helpful information for distinguishing palmoplantar eczematous lesions and psoriatic lesions as well as finding pathogenesis mechanism and furthermore evaluation of targeted therapies especially for VEGF receptors (Canavese *et al*., 2010).

MATERIALS AND METHODS

In this cross-sectional study, patients with primary clinical diagnosis of plantopalmar psoriasis, eczema, and lichen planusreferring to Razi Skin Hospital, Tehran, Iran, from October 2014 to 2015, were enrolled. After confirmation of diagnosis, patients entered this study.

The inclusion and exclusion criteria for entry of this study were:

- All the selected patients aged above 18
- Only patients with palmoplantar chronic plaque were enrolled
- The selected patient were otherwise normal
- All patients stopped topical and systemic treatment at least 2 weeks before taking biopsy.

Histopathological Evaluation of Specimen

Hematoxylin and eosin (H and E) stained slides were collected from archives of pathology department, razi hospital, Tehran, Iran and all cases were reviewed to verify the histologic findings. Representative paraffin-embedded tissue blocks were selected for VEGF, Ki67, and GLUT-1 immunohistochemistry stainings. Histological criteria for diagnosis of Chronic Plaque Psoriasis included Skin lesions with confluent parakeratosis with downward vertical elongation of rete ridges, hypogranulosis, suprapapillary plate thinning, Munro's microabscesses, spongiform (kogoj) postules and tortous vessels (Figure 1) Histological criteria for diagnosis of Eczematous lesions included skin lesions with hypergranulosis, irregular acanthosis, spongiosis, areas of hyperkeratosis and parakeratosis (Figure 2) (Kamyab Hesari *et al*., 2014). A study found that the presence of multiple parakeratotic foci, placed vertically and alternating with orthohyperkeratosis favored of psoriasis. Psoriasis in this site often shows spongiosis, spongiotic vesicles were present in 75% of cases in this series.neutrophils and/or plasma are often present in parakeratotic layers in palmoplantar psoriasis. (MD JWP, 2015) Regarding pathophysiological aspect, lichen planus is characterized by abnormally increased proliferation and differentiation of keratinocytes which shows increased cellular turnover rate than normal skin while turnover time is markedly shortened in psoriasis. (Weinstein *et al*., 1965; Kose *et al*., 2007) In this study we selected lichen planus as control group to evaluate the proper functioning of immunohistochemical markers.

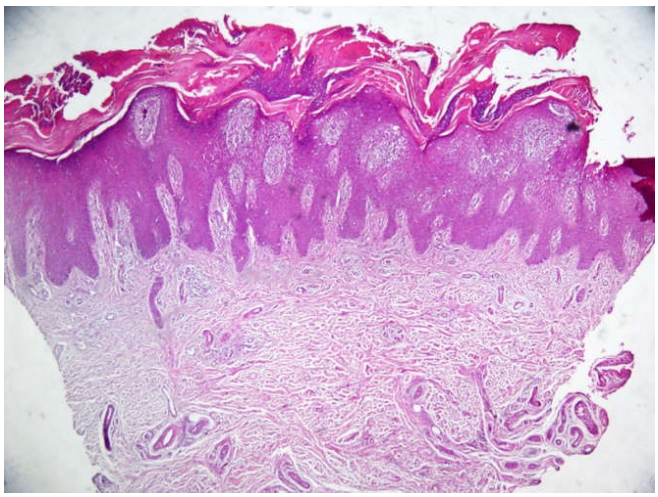


Figure 1. Psoriasis, H&E staining (magnification:100X)

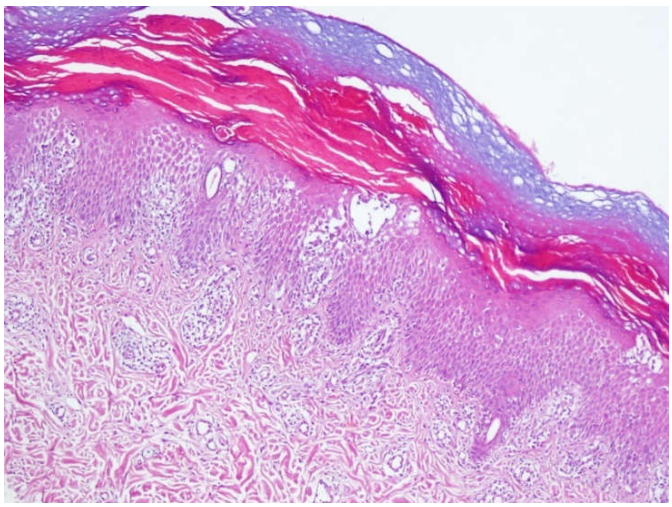


Figure 2. Eczematous lesion, H&E staining (magnification: 200X)

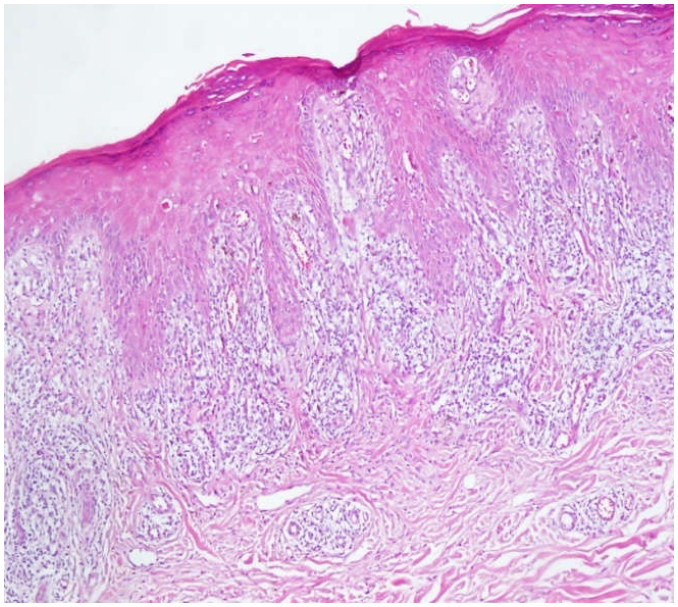


Figure 3. Lichen planus, H&E (magnification:200 X)

Immunohistochemical staining method

Paraffin sections prepared from psoriatic, eczematous, and lichen planus lesions were incubated overnight with either a polyclonal VEGF antibody (Dako), Ki67 antibody (Dako) and GLUT1 antibody (Abcam) and incubation was done using a HRP conjugated anti rabbit antibody (Dako). Staining was visualized using DAB method.

Interpretation of immunohistochemical markers

GLUT-1: membranous or cytoplasmic staining patterns in any number of cells were considered positive.

Intensity: the intensity of staining was categorized into mild, moderate, and severe.

Distribution: Distribution of patterns divided into focal, discontinuous, and diffuse.

Localization: Lesions with positive immunostaining further divided into four categories regarding location of the lesion, basal, suprabasal, both (basal and suprabasal), and full thickness.

Ki-67: Any number of cells with nuclear and nucleolar expression were considered positive, and the percentage of positive cells in relation to the epidermis was calculated. Specimen were also examined for positivity, intensity, distribution, and localization of pathologic findings with the same criteria for GLUT-1 IHC interpretation.

VEGF: Specimen first were evaluated for positivity for cytoplasmic staining pattern, and then presence of vascular proliferation and ectasia.

Vascular proliferation categorized as bellow:

1+ defined as weak staining
2+ defined as moderate staining
3+ defined as strong staining

Statistical analysis

Statistical analysis performed using SPSS version 16.0.1 (SPSS Inc., Chicago, IL, U.S.A.). Chi2 and Fischer-exact tests were used to compare qualitative variables and P=< 0-05 was considered significant.

RESULTS

Demographic data: Forty five cases ranging from 18 to 60 year old including 15 cases of palmoplantar psoriasis, 15 cases of eczema of palms and soles enrolled into our study. Also 15 cases of lichen planus were included in the study to represent the control group.

	Male	Female
Psoriasis	7	8
Eczema	5	10
Lichen planus	9	6

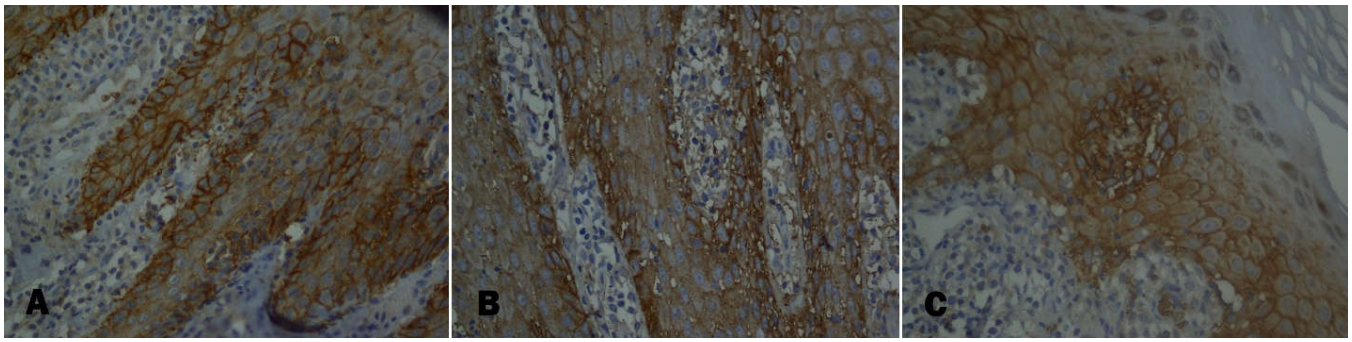
Expression of GLUT-1 in Psoriasis, eczema, and lichen planus

The histopathological and immunohistochemical data of the patients are illustrated in tables bellow:

GLUT-1 was expressed all the cases from three groups. According to intensity lesions categorized in 3 groups of mild, moderate, and severe. Most of the cases from three groups showed moderate intensity, and there were no significant

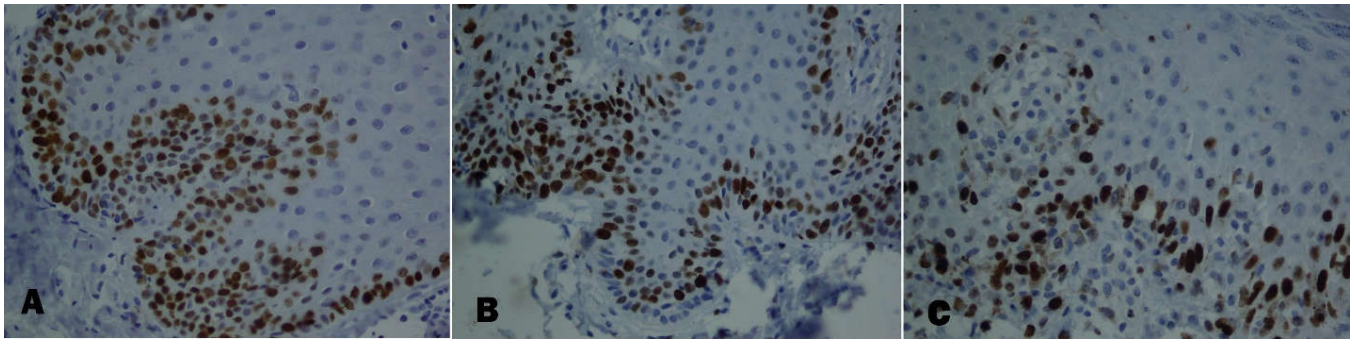
GLUT1		Psoriasis	Eczema	Lichen Planus (control)	Significance P-value (three groups)	Significance P-value (psoriasis and eczema)
Expression	Positive	15(100%)	15(100%)	15(100%)		
	Negative	0	0	0		
Intensity	Mild	3(20%)	1(6.7%)	2(13.3%)	P=0.587	P=0.291
	Moderate	9(60%)	13(86.7%)	11(73.3%)		
	Severe	3(20%)	1(6.7%)	2(13.3%)		
Localization	Basal	7(46.7%)	6(40%)	1(6.7%)	P=0.102	P=1.000
	Suprabasal	0	0	.		
	both	1(6.7%)	2(13.3%)	4(26.7%)		
	Full-thickness	7(46.7%)	7(46.7%)	10(66.7%)		
Distribution	Focal	0	0	0	P=0.020	P= 0.008
	Discontinous	6(40%)	13(86.7%)	11(73.3%)	(Eczema and Psoriasis 0.008)	
	Diffuse	9(60%)	2(13.3%)	4(26.7%)		

Figure 4. GLUT-1 expression, A) Psoriasis B) Eczema C) Lichen planus· x200.All 3 groups positive for GLUT1



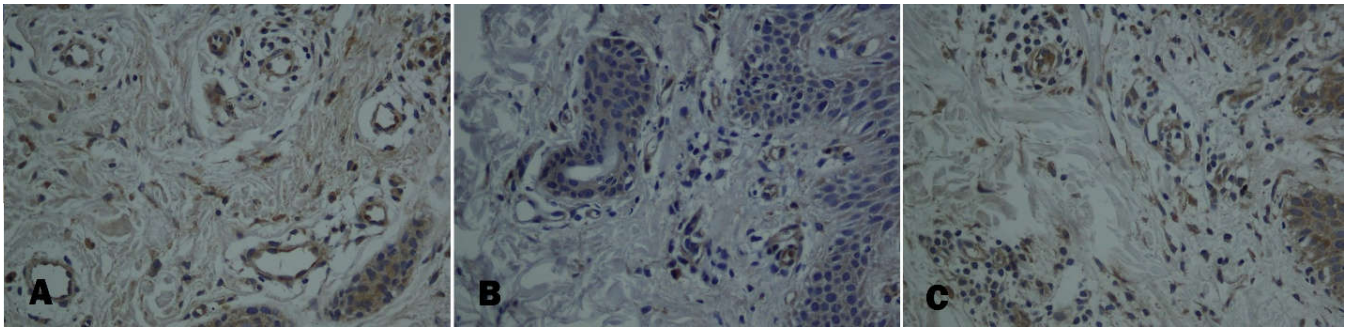
Ki-67		Psoriasis	Eczema	Lichen Planus (control)	Significance P-value (three groups)	Significance P-value (psoriasis and eczema)
Expression	Positive	15(100%)	15(100%)	15(100%)		
	Negative	0	0	0		
Intensity	Mild	0	0	0	P=0.007	
	Moderate	0	0	5(33.3%)		
	Severe	15(100%)	15(100%)	10(66.7%)		
Localization	Basal	11(73.3%)	11(73.3%)	14(93.3%)	P=0.340	P=0.447
	Suprabasal	0	0	0		
	both	4(26.7%)	4(26.7%)	1(6.7%)		
	Full-thickness	0	0	0		
Distribution	Focal	2(13.3%)	2(13.3%)	2(13.3%)	P=0.762	P=0.659
	Discontinous	8(53.3%)	11(73.3%)	9(60%)		
	Diffuse	5(33.3%)	2(13.3%)	4(26.7%)		

Figure 5. Expression of Ki-67 : A) Psoriasis B) Eczema C) Lichen planus (x400 for fig A & B, x100 for fig C) All 3 groups show severe positive staining for ki67



VEGF		Psoriasis	Eczema	Lichen Planus (control)	Significance P-value (three groups)	Significance P-value (psoriasis and eczema)
Expression	Positive	15(100%)	15(100%)	15(100%)		
	Negative	0	0	0		
Proliferation	1+	1(6.7%)	11(73.3%)	13(86.7%)	P-value= 0.0001	P=0.0001
	2+	12(80%)	4(26.7%)	2(13.3%)		
	3+	2(13.3%)	0	0		
Ectasia	Positive	14(93.3%)	10(66.7%)	4(25.7%)	p-value= 0.001	P=0.169
	Negative	1(6.7%)	5(33.3%)	11(73.3%)		

Figure 6. VEGF expression in: A) Psoriasis B) Eczema C) Lichen planus x400



difference between 3 groups. Assessment of localization showed full-thickness involvement in majority of the cases. GLUT-1 distribution analysis revealed discontinuous pattern in most of the cases of eczema and lichen planus but in psoriasis cases stained diffusely in 9 out of 15 cases (P-value: 0.02). Comparing GLUT-1 distribution of psoriasis and eczema showed a p-value of 0.008 which was significant. (Figure 4)

The Relationship Between localization and Intensity of Ki-67 Expression in Psoriatic Lesions, eczematous lesions and Lichen planus

ki67 was expressed all the cases from 3 groups. All of the cases of psoriasis and eczema showed severely intense expression of Ki-67 but 5 out of 10 cases of lichen planus stained moderately intense. Regarding localization and distributaion there were no significant difference between three examined groups.

Expression of VEGF in Psoriasis, eczema, and lichen planus

VEGF expression was detected in all of the examined groups. Proliferation index of this marker was significantly higher in psoriasis and most of which were 2+ positive; on the other hand, eczema and lichen planus had less vascular proliferation (p-value= 0-0001). Vascular ectasia was positive in majority of cases in both psoriasis and eczema with no significant

difference (p-value=0.169) but most of the specimen with diagnosis of lichen planus showed no vascular ectasia (p-value=0.001).

DISCUSSION

Differentiating psoriasis from eczematous dermatitis in palmoplantar region is challenging since both conditions share several common overlapped histomorphologic features specially in non-typical cases. (Kamyab Hesari *et al*., 2044) In the presented study, we evaluated the utility of three markers of GLUT-1, Ki-67, and VEGF in palmoplantar psoriasis, eczema, and lichen planus in the mentioned region, and we aimed to investigate immunohistochemical markers to differentiate these lesions. GLUT-1 (glucose transporter 1) is most frequent glucose transporter which acts as facilitator of glucose transport across cell membranes. Previous studies shows that this transporter upregulates in highly proliferating cells with high glucose demand such as tumoral cells as well as benign lesions with excessive cell proliferation such as Psoriasis. In our study, there was no significant difference between GLUT1 expression in 3 examined groups. Another study showed upregulation of GLUT-1 in basal layer of the psoriatic skin which facilitates keratinocyte proliferation. (Abdou *et al*., 2013) Our study showed no significant difference for GLUT1 intensity & localization between chronic plaque of psoriasis and eczema as well as lichenplanus.In the present study we have observed that most cases of psoriasis stainedin diffuse distribution for GLUT1 (60%) compared with eczema (13.3%) and lichen planus (26.7%). On the other hand most cases of eczema show discontinuous staining for GLUT1 marker (86.7%). These findings may indicate higher glucose demand of psoriatic and eczematous lesions comparing lesions of lichen planus also higher expression of GLUT-1 can play role in facilitation of keratinocyte proliferation. (Tao *et al*., 2008) Ki-67 is a universal marker of proliferative activity. Higher expression of Ki-67 in psoriatic lesions is associated with clinical severity of the disease. Amin and Azim, 2012; Sezer *et al*., 2015) In this study there was no significant difference between the 3 groups regarding Ki-67 expression localization and distribution but severity of Ki-67 expression was higher in psoriasis (100%) and eczema (100%) comparing lichen planus (10%), although there was no significant difference between psoriasis and eczema. Other studies showed the same results meaning both epidermis of involved and uninvolved skin of psoriasis showed higher Ki-67 expression in comparison with normal skin. (Abdou *et al*., 2013) One study showed association of higher nucleolar pattern with progressive keratinocyte proliferation in psoriasis. (Abdou *et al*., 2013) Angiogenesis is regarded as a hallmark for psoriasis, and also in eczematous lesions. Previous studies emphasized on increased angiogenesis in psoriatic lesions, using vascular markers such as CD34 and VEGF in order to discover a therapeutic approach in psoriasis. Overexpression of CD34 was seen in psoriatic lesions in one study. (Amin and Azim, 2012) VEGF overexpression has been investigated in several studies which has been led to postulation of VEGF-antagonist therapy in psoriatic patients. (Marina *et al*., 2015; Canavese *et al*., 2010; Simonetti *et al*., 2006) We used VEGF for evaluating vascular proliferation and vascular ectasia in our three examined groups. In our study VEFG expressed in 3 groups and vascular proliferation was significantly higher in psoriatic lesions comparing lichen planus and eczematous lesions so that most cases of psoriasis show moderate dermal vascular proliferation (80%) comparing most cases of eczema (73%) and lichen planus (86%) which show mild vascular proliferation. In this manner only cases of psoriasis (13.3%) show sever dermal vascular proliferation. Also in our study, vascular ectasia is significantly higher in psoriasis (93.3%) and eczema (66.7%) comparing lichen planus (25.7%). Other studies demonstrated that in chronic plaque psoriasis there is a significant association between Ki-67 expression and endothelial proliferation and expression of markers of angiogenesis (CD34) (Amin and Azim, 2012) Regarding mentioned findings utilization of VEFG is useful for differentiating palmoplantar psoriasis from eczema. Also pattern of distribution for GLUT1 staining may be helpfull. Differentiating these two conditions can help clinicians to assess the prognosis and better management the affecting patients. Although sometimes the therapies for both conditions are overlapped. (De Rie *et al*., 1995)

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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