Araştırma Makalesi
Research Article

The Use of GLUT-1, Ki-67 and PCNA Antibodies as Immunohistochemical Markers in Histopathological Differential Diagnosis of Psoriasis and Chronic Spongiotic Dermatitits

PSORİAZİS VE KRONİK SPONGİOTİK DERMATİTLERİN HİSTOPATOLOJİK AYIRICI TANISINDA İMMÜNHİSOHİSTOKİMYASAL OLARAK GLUT-1, Ki-67 VE PCNA ANTİKORLARININ KULLANIMI

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ABSTRACT

Introduction: The present study aims to investigate the benefits of the immunohistochemical antibodies of glucose transporter 1 (GLUT-1), the nuclear protein Ki-67, and proliferating cell nuclear antigen (PCNA) to distinguish between psoriasis and chronic spongiotic dermatitits.

Materials and Methods: We evaluated 32 cases of psoriasis and 35 cases of spongiotic dermatitis that had been clinicopathologically diagnosed. Skin tissue from reduction mammoplasty procedures was used as a control group. Skin biopsy sections stained with H&E were examined. Additional immunohistochemistry was performed, including Ki-67, PCNA and GLUT-1. Histological findings were also noted

Results: There was no significant difference between psoriasis and chronic spongiotic dermatitits groups in the results of Ki-67 or PCNA staining. GLUT-1 distribution was also similar; however, there was a difference between the groups concerning GLUT-1 intensity. The percentage of cases with moderate GLUT-1 staining was higher in the psoriasis group, whereas the percentage of strong staining was higher in the chronic spongiotic dermatitits group. Most of the examined histopathological features of psoriasis and chronic spongiotic dermatitits cases were different.

Conclusion: The intensity of GLUT-1 staining with the appropriate histopathological and clinical findings may have a limited benefit in the differential diagnosis of psoriasis and chronic spongiotic dermatitits.

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Keywords: chronic spongiotic dermatitits, glucose transporter 1, immunohistochemistry, Ki-67, proliferating cell nuclear antigen, psoriasis

ÖZ

Amaç: Bu çalışma, glukoz transporter 1 (GLUT-1), nükleer protein Ki-67 ve proliferatif hücre nükleer antijeni (PCNA) immünohistokimyasal antikorlarının, psoriasis ve kronik spongiotik dermatitlerin ayırıcı tanısında faydalarını araştırmayı amaçlamaktadır.

Gereç ve Yöntem: Klinikopatolojik olarak tanısı konulmuş 32 adet psoriazis ve 35 adet spongiotik dermatit vakası değerlendirildi. Redüksiyon mamoplasti materyallerinin üzerindeki deri dokusu kontrol grubu olarak kullanıldı. H&E ile boyanan deri biyopsileri tekrar incelendi. Ayrıca Ki-67, PCNA ve GLUT-1 dahil olmak üzere ek immünohistokimyasal inceleme yapıldı. Histolojik bulgular da ayrıca not edildi.

Bulgular: Psoriasis ve kronik spongiotik dermatit grupları arasında, Ki-67 ve PCNA boyama sonuçları arasında anlamlı fark yoktu. GLUT-1 dağılımı da iki grup arasında benzerdi; ancak GLUT-1 yoğunluğu açısından gruplar arasında fark vardı. Orta derecede GLUT-1 boyaması olan vakaların yüzdesi psoriazis grubunda daha yüksek iken, güçlü boyanma yüzdesi olan vakalar kronik spongiotik dermatit grubunda daha fazlaydı. İncelenen histopatolojik özelliklerinin pek çoğu psoriasis ve kronik spongiotik dermatit vakalarında anlamlı olarak fark gösteriyordu.

Sonuç: Uygun histopatolojik ve klinik bulgularla birlikte GLUT-1 boyamasının yoğunluğu, psoriazis ve kronik spongiotik dermatitlerin ayırıcı tanısında sınırlı bir fayda sağlayabilir.

Anahtar Sözcükler: kronik spongiotik dermatit, glukoz transporter 1, immünohistokimya, Ki-67, proliferating cell nuclear antigen, psoriazis

Psoriasis is a chronic dermatosis that affects approximately 1-2% of the population. Frequently affected areas are the elbows, scalp, lumbosacral region, intergluteal sulcus, and the glans penis. The classic skin lesion of psoriasis vulgaris is a silvery, scaly, well-demarcated lesion, which is present in approximately 85-90% of the patients. Classic microscopic features observed in the histopathological examination are prominent epidermal thickening, a regular extension of the rete ridges, and loss of the granular layer, suprapapillary epidermis thinning, parakeratosis, spongiform micropustules, and Munro's microabscesses (1).

Contact dermatitis is a significant type of spongiotic dermatosis. Allergic contact dermatitis and irritant contact dermatitis are common. In the early stages, intense inflammation and eosinophilia, spongiotic vesicles, lymphocytes, macrophages and Langerhan's cells can be seen histopathologically. However, in long-term lesions, spongiosis reduces and psoriasiform epidermal hyperplasia develops. In this period of the disease, it may be challenging to distinguish chronic spongiotic dermatitits from psoriasis histopathologically (1-2). In addition, patients with insufficient clinical knowledge, isolated lesions in the region (palmoplantar or scalp), and changes due to previous treatment may complicate the differential diagnosis of psoriasis and chronic spongiotic dermatitits based on histological data.

The typical histological patterns are often lacking in the palmoplantar localization and that these two conditions have similar histological features, such as acanthosis, parakeratosis and spongiosis (3). Similarly, isolated involvement of the scalp requires clinical data and careful histological examination, which may also lead to difficulties in the differential diagnosis (4).

Another factor that makes it challenging to diagnose these conditions using histological findings is the treatment effect. In psoriasis patients, treatment may result in changes to the extent of parakeratosis, the number of mitosis, granulocyte, and mononuclear cells, as well as Munro's microabscesses, and the papillary vascular morphology (5, 6). It has been proposed that the examination of immunohistochemical antibodies in addition to histological findings may be helpful in the diagnosis of psoriasis (7). Research is ongoing to find a reliable immunohistochemical marker for distinguishing psoriasis from chronic spongiotic dermatitits.

In a recent study on metabolic pathways that control skin hemostasis and inflammation, it was stated that keratinocytes and T cells actively metabolize the nutrients in their microenvironment and this is important in inflammatory skin diseases. In this study, it has been suggested that Glut-1 and glycolysis may be the targetted in psoriasis and atopic dermatitis, although relevance of glycolysis versus oxidative phosphorylation is not reported (8). Glucose transporter 1 (GLUT-1) is the most common glucose-carrier protein in humans. In normal skin, GLUT-1 is expressed in the basal and suprabasal layers. The immunoreactivity found in the basal layer and under the suprabasal layer, decreases in the upper layers of the epidermis (9). However, it has also been reported that the expression level of GLUT-1, which has a function related to cell energy needs, is completely negative in normal skin (10).

The primary pathological changes observed in psoriasis are epidermal hyperproliferation with abnormal differentiation, angiogenesis, and dermal inflammation. It is thought that the expression of GLUT-1 may increase in patients with psoriasis (10, 11). It has also been suggested that GLUT-1 may be associated with disease severity (12).The nuclear protein Ki-67 is a well-known cell proliferation marker. Positive staining with Ki-67 is obtained only on the basal layer in normal skin. However, samples from psoriasis patients, are highly expressed in lesioned skin and at a lesser rate in lesionless skin areas compared with normal skin (7). Decreased Ki-67 rates due to treatment suggested that Ki-67 may also be associated with disease severity (13).

Proliferating cell nuclear antigen (PCNA) is a protein that has been demonstrated to act as a promoting factor for DNA polymerase and is required for initial DNA synthesis and replication in all eukaryotic organisms. Many studies have been conducted to investigate the staining properties of PCNA as an indicator of keratinocyte proliferation in dermatological diseases. Increased proliferation of PCNA is observed in psoriasis, ichthyosis, chronic dermatitis and verruca vulgaris (14-16).

This study was an investigation of the usefulness of the immunohistochemical antibodies of GLUT-1, Ki-67, and (PCNA in) the differential diagnosis of psoriasis and chronic spongiotic dermatitits.

MATERIALS AND METHODS

Case Selection and Clinical Data

Formalin-fi fixed, paraffin-embedded blocks prepared for 32 patients with psoriasis and 35 patients with chronic spongiotic dermatitits, as well as 52 tissue samples from reduction mammoplasty patients were used in this study. Details of patient age and sex, lesion localization, and lesion prevalence were recorded.

Histological Analysis

The hematoxylin-eosin stained preparations of this study cases were re-evaluated. Hyperkeratosis, parakeratosis, acanthosis, granular layer status, necrotic keratinocyte count, basal cell vacuolization, exocytosis, epidermal edema, suprapapillary epidermal thickness, inflammatory response, mitosis count, superficial capillaries, and the presence of Munro's microabcesses, and Kogoj's spongiform pustules were assessed.

Immunohistochemical Study

The paraffin blocks were cut into 4-micron slices and mounted onto poly-L-lysine-coated slides to improve the adhesion of the tissue. The sections were incubated in a 60˚C oven for onehour and then deparaffinized with xylol for 15 minutes. The slides were hydrated through graded alcohols to deionized water.Ki-67 Monoclonal Antibody (SP6) (Thermo Fisher Scientific, Inc., Waltham, MA, USA) was used as the primary antibody for Ki-67 at a dilution of 1:200, PCNA Antibody (PAS-3251), a rabbit polyclonal antibody for PCNA (Thermo Fisher Scientific, Inc., Waltham, MA, USA)was used at 1:400 dilution, and GLUT-1 Monoclonal Antibody (SPM498)for GLUT-1 (Thermo Fisher Scientific, Inc., Waltham, MA, USA) at 1:200 dilution and incubated for one hour at 37°C. Staining was performed with a Ventana BenchMark XT automatic immunohistochemistry device (F. Hoffmann-La Roche Ltd., Basel, Switzerland). The preparations were passed through a gradually increasing alcohol concentration for fiveminutes (96% and 99.5%) and then held in xylol for 15 minutes. The prepared sections were examined under a light microscope and the results were evaluated by two pathologists.

Evaluation of GLUT-1 Staining

The staining intensity of GLUT-1 was categorized as weak (pale), mild, or strong (dark). The distribution of staining was classified as staining only in the basal layer, basal and suprabasal focal staining, or staining of the entire epidermis (Figure 1, 2, 3). Weak and strongly stained cell numbers/10HPF (high power field) were noted (Figure 4).

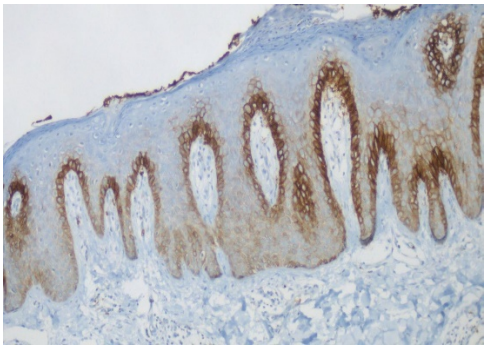

Figure 1: Basal and focal suprabasal weak staining withGLUT-1 (X 400)

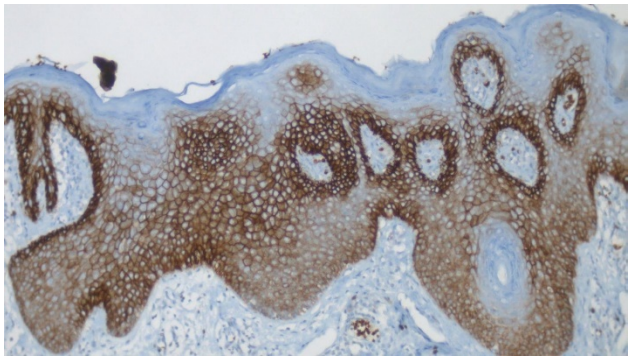

Figure 2: Suprasal, diffuse and modarate staining with Glut-1 (x400)

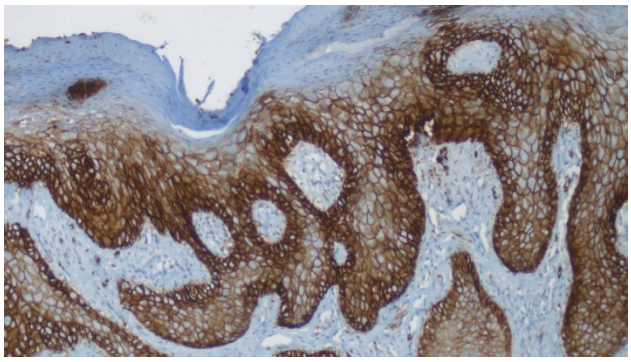

Figure3: Suprabasal, diffuse and strong staining with GLUT-1 (X 400)

Evaluation of Ki-67 and PCNA Staining

Weak and strongly stained cell numbers/10HPF (high power field) were noted (Figure 4).

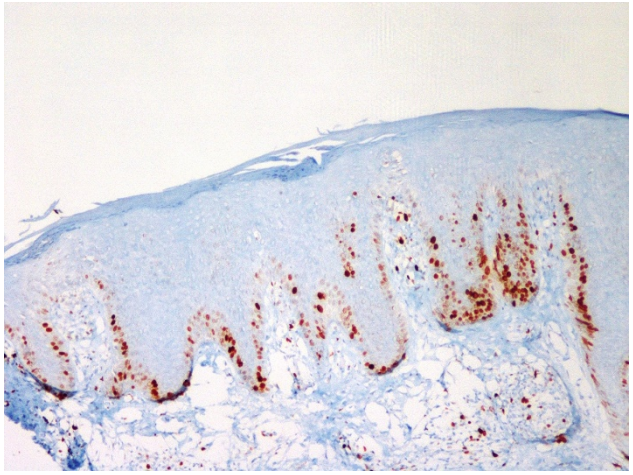

Figure 4: Basal and subrabasal staining with Ki-67 (x400)

Statistical Analysis

SPSS for Windows, Version 15.0software (SPSS Inc., Chicago, IL, USA) was used to evaluate the data. Qualitative data were described using the number and percentage. Quantitative data were described using the range, mean, SD, and median. Comparisons of categorical variables between different groups were conducted using a chi-square test. Normal distribution was evaluated with a Shapiro-Wilk test, histogram, histogram and a Q-Q plot. The Kruskal-Wallis test (Ki-67 and mitosis)and one-way analysis of variance (other data) were used for between-group comparison. Multiple comparison testing was performed with the Dunn-Bonferroni and Tukey tests. $P<0.05$ was considered significant. The Erciyes University Clinical Research Ethics Committee approved this study (No:2017/488; Date 27.10.2017).

RESULTS

The mean age of the study participants was 43.7 years in the chronic spongiotic dermatitits group, 39.8 years in the psoriasis group, and 42.1 years in the control group. There was no significant difference between the age values of the groups. The female: male ratio was 1: 1.19 in the chronic spongiotic dermatitits group and 2.2: 1 in the psoriasis group. Analysis of the presence and severity of hyperkeratosis, presence and regularity of acanthosis, decrease in the granular layer, presence of basal cell vacoulization, suprapapillary epidermal thinning, and a variety of inflammatory cells revealed statistically significant differences between psoriasis and chronic spongiotic dermatitits groups. Kogoj's spongiform pustules and Munro's microabscesses were the most important findings for psoriasis cases. There were no significant differences in the mitosis, lymphocyte exocytosis, intracellular edema, spongiosis, or necrotic keratinocyte results.

Evaluation of Immunohistochemical Staining

The immunohistochemical results can be seen in Table 1-3.

GLUT-1 Staining Results

GLUT-1 basal staining was seen in five (13%) cases of chronic spongiotic dermatitits, six (18%) cases of psoriasis, and one (1.9%) case in the control group. Suprabasal focal staining was present in 19 (54%) cases of chronic spongiotic dermatitits, 14 (43%) cases of psoriasis, and 12 (37%) cases in the control group. Staining of the entire epidermis was present in 11 (31%) cases of chronic spongiotic dermatitits, 12 (37.5%) cases of psoriasis, and 18 (34%) cases in the control group. The distribution of GLUT-1 did not differ significantly between the groups ($p=0.579$) (Table 1).

Table 1. Comparison of immunohistochemical properties (GLUT-1)

Variables		Groups			p-value
		Chronic Spongiotic Dermatitits n (%)	Psoriasis n (%)	Control n (%)	
GLUT-1 severity	Mild	14(40)	7(21.9)	20(38.5)	<0.001
	Moderate	11(31.4)	20(62.5)	32(61.5)	
	Strong	10(28.6)	5(15.6)	0(0)	
GLUT-1 prevalence	Basal	5(14.3)	6(18.8)	1(1.9)	0.091
	Suprabasal, focal	19(54.3)	14(43.8)	12(37.5)	
	Suprabasal, diffuse	11(31.4)	12(37.5)	18(34.6)	

GLUT-1 values are given as numbers and percentages. (GLUT-1: Glucose transporter 1)

GLUT-1 Staining Results

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Weak (pale) staining with GLUT-1 was observed in 7 (21.9%) cases in the psoriasis group, 14 (40%) cases in the chronic spongiotic dermatitits group, and 20 (38%) cases in the control group. There was mild staining in 20 (62%) cases in the psoriasis group, 11 (31%) cases in the chronic spongiotic dermatitits group, and 32 (61%) cases in the control group. Strong (dark) staining was seen in 10 (28%) in the psoriasis group and five (15%) in the chronic spongiotic dermatitits group. There was a significant difference between the groups in GLUT-1 staining intensity (p<0.001). The psoriasis and chronic spongiotic dermatitits groups were also significantly different (p=0.039) (Table 1).

Evaluation Of Ki-67 Staining

The median strong (dark) stain Ki-67 cell count was seven in the chronic spongiotic dermatitits group, 9.5 in the psoriasis group, and six in the control group. There was a significant difference between psoriasis group and the control group (p=0.026). Pale staining with Ki-67 had a median cell count of 32 in the chronic spongiotic dermatitits group, 37.5 in the psoriasis group, and 13 in the control group. There was no significant difference between psoriasis and chronic spongiotic dermatitits groups (p=0.218), but there was a significant difference in pale staining when compared with the control group (p<0.001). The median value of the total Ki-67 cell count was 46.5 in the psoriasis group, 41 in the chronic spongiotic dermatitits group, and 20 in the control group. While there was no Significant difference between psoriasis and chronic spongiotic dermatitits groups (p=0.204), there was a significant difference in staining in the control group (p<0.001) (Table 2).

Table 2. Comparison of immunohistochemical properties (Ki-67)

Variables	Groups			p-value
	Chronic Spongiotic Dermatitits (median and 25-75 percentiles)	Psoriasis (median and 25-75 percentiles)	Control (median and 25-75 percentiles)	
Ki-67	Total 41(25-48)	46.5(35.2-64.5)	20(14-27)	<0.001

Ki-67-stained cell numbers/10HPF (high power field) (median and 25-75 percentiles)

To summarize, there was no significant difference in pale staining, dark staining, or total Ki-67 values between psoriasis and chronic spongiotic dermatitits groups.

Evaluation of PCNA Staining

The mean value of strong (dark) staining with PCNA was 19.28 in chronic spongiotic dermatitits group, 19.21 in the psoriasis group, and 15.13 in the control group. The mean value of pale staining was 36.85 in the chronic spongiotic dermatitits group, 38.65 in the psoriasis group, and 31.5 in the control group. When the three groups were evaluated together, there was a small difference between dark (p=0.08) and pale (p=0.06) staining, but this difference was not statistically significant. However, when the total numbers were compared, this difference was significant (p<0.001).

There was no significant difference between psoriasis and chronic spongiotic dermatitits groups concerning pale (p=0.237), dark (p=0.131), or total (p=0.074) staining (Table 3).

Table 3. Comparison of immunohistochemical properties (PCNA)

Variables		Groups			p-value
		Chronic Spongiotic Dermatitits	Psoriasis	Control	
PCNA	Total	53.85±11.80	58.18±10.99	46.19±14.74	<0.001

Numerical values shown for PCNA are mean and SD. (PCNA: Proliferating cell nuclear antigen)

DISCUSSION

Various results have been reported in studies investigating roles for immunohistochemical markers in the differential diagnosis of chronic spongiotic dermatitits and psoriasis. This study aims to investigate the benefits of the immunohistochemical antibodies of glucose transporter 1 (GLUT-1), the nuclear protein Ki-67, and proliferating cell nuclear antigen (PCNA) to distinguish between psoriasis and chronic spongiotic dermatitits. However this article has some limitations. It is known that the microanatomy of the skin varies from one region of the body to another. In this study, the effect of localization of lesions on immunohistochemical staining and histological parameters was not evaluated. Using the skin on the breast

instead of similar anatomical localization of lesions as the control group is another limitation of the study. Also, while evaluating the staining intensity, it was not taken into account that the staining quality may change according to the year of biopsy. One of the strengths of this study is that ki-67 was evaluated with PCNA, a nuclear marker. Other strengths of the study are that the study included a control group and the patients' clinical follow-up information was accessed.

Increased expression of GLUT-1 in psoriasis patients has been suggested in some studies. In one study, no positive staining with GLUT-1 was observed in the control group, but a statistically significant difference was found in the lesional and non-lesioned skin of psoriasis patients (10). A similar study was conducted by Tao et al. (11), who compared normal skin with psoriatic skin and found a statistically significant over-expression of GLUT-1 in psoriasis patients. However, in Tao et al.'s (11) study, the findings showed that GLUT-1 could be weakly stained in a normal epidermis sample, in contrast to the findings of Abdou et al. (10).

The staining intensity of GLUT-1 is generally different at the basal and suprabasal layers. In this study, GLUT-1 stained strongly (dark) in the basal layer of all of the biopsies. Therefore, the intensity of GLUT-1 was evaluated according to the strength of suprabasal staining. In our study, the intensity of GLUT-1 did not differ between the groups and positive staining of the entire epidermis was seen in the control group. The GLUT-1 intensity was different between the groups: Most of the strongly stained cases were in the chronic spongiotic dermatitits group and most of the cases with moderate staining were in the psoriasis group. Most of the cases in the control group had mild or moderate staining.

GLUT-1 expression can be expected to increase in many dermatoses where cell energy needs to increase. In addition, GLUT-1 can be affected by the severity of diseases, which limits its usefulness in a differential diagnosis. There are several reports suggesting that Ki-67 staining will support the diagnosis of psoriasis. In the study performed by Amin et al. (7), there was a difference in the Ki-67 index between the lesional skin and non-lesioned skin of psoriasis patients and the control group. In a similar study, Abdou et al. (10) found no difference between lesional skin and non-lesioned skin of psoriasis patients; however, there was a high rate of positivity compared with the control group. Sezer et al. (17) compared a psoriasis group and a non-psoriasis psoriasiform dermatitis group of patients with pityriasis rubra pilaris, pityriasis rosea, and lichen simplex diagnoses in a suprabasal evaluation, and the total Ki-67 proliferation was significantly higher in the psoriasis group. In our study, no significant difference was found in the Ki-67 pale staining, strong staining, or total staining values between psoriasis and chronic spongiotic dermatitits groups. The control group demonstrated significantly less staining.

PCNA is a marker that can be used with or instead of Ki-67. Jung et al. (18) found that these two markers hadvery strong correlations. In the same study, increased staining in psoriasis was observed. Kanitakis et al. (15) reported higher PCNA values in the psoriasis group than the control group. They observed that the increase in PCNA in cases of chronic spongiotic dermatitits was not very high. Similar to psoriasis, PCNA staining rates were significantly higher in atopic dermatitis and verruca vulgaris tissue. Another point highlighted in Kanitakis et al.'s study was that PCNA staining might be related to treatment resistance.

In our study, weak and strongly stained cell numbers were noted. We found no significant difference in the PCNA staining between the chronic spongiotic dermatitits and psoriasis groups. The control group revealed significantly less staining. In conclusion, our findings suggest that PCNA and Ki-67 staining did not help in the differential diagnosis of chronic spongiotic dermatitits and psoriasis. Although there was darker staining with GLUT-1 in the chronic spongiotic dermatitits group, and this might provide some benefit, clinical and histological data continue to be more significance in the differential diagnosis.

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