Mast Cells and p53 Expression in Psoriasis Vulgaris

Psoriazis Vulgariste Mast Hücreleri ve p53 Gen Ekspresyonu

Figen BARUT, MD,[a]
Sibel BEKTAŞ, MD,[a]
Banu DOĞAN GÜN, MD,[a]
Burak BAHADIR, MD,[a]
Şükrü Oğuz ÖZDAMAR, MD[a]

[a]Department of Pathology, Zonguldak Karaelmas University Faculty of Medicine, Zonguldak

Geliş Tarihi/*Received:* 24.10.2009
Kabul Tarihi/*Accepted:* 05.06.2010

This study was presented as poster in 21st European Congress of Pathology, 8-13 September 2007, Istanbul, TURKEY.

Yazışma Adresi/*Correspondence:*
Figen BARUT, MD
Zonguldak Karaelmas University Faculty of Medicine,
Department of Pathology, Zonguldak, TÜRKİYE/TURKEY
figenbarut@yahoo.com

ABSTRACT Objective: Psoriasis vulgaris is a chronic inflammatory T-cell mediated immune dermatosis characterized by a high epidermal cell turnover, which results in a typical epidermal hyperplasia. The aim of this study is to show the correlation between p53 protein accumulation that provides epidermal hyperproliferation and mast cell count in the pathogenesis of psoriasis vulgaris. **Material and Methods:** In this study, punch biopsies from 56 psoriasis cases have been evaluated. The presence of mast cells in with metachromatic granules are with 1% toluidin blue stain solution has been investigated. In addition, mast cells that show immunoreactions to "mast cell tryptase" antibody have been observed and proportion of p53 staining in epidermal keratinocytes has been determined. **Results:** In psoriasis cases, mast cells count (mean) and proportion of p53 staining (mean) were 69.3 ± 21.5/mm^2 and 602.3 ± 192.1 respectively, in the group without any treatment (n: 20); 57.1 ± 27.5/mm^2 and 373.1 ± 193.6 respectively in the group treated with psolaren plus UVA radiation (n: 36). In the control group, it was demonstrated that mast cells count was 74.6 ± 25.9/mm^2 (mean) and proportion of p53 staining was 176.4 ± 109.2 (mean). No correlation has been found between mast cell count and proportion of p53 staining in psoriasis groups. However, a moderate correlation (r= 0.30 P< 0.05) has been found between mast cell count and proportion of p53 staining in the control group. **Conclusion:** Since the correlation obtained between mast cell number and proportion of p53 staining in the healthy skin was not observed psoriasis cases, it can be thought that these variables take part in different pathways of pathogenesis.

Key Words: Psoriasis; mast cells; p53 protein (325-355)

ÖZET Amaç: Psoriazis vulgaris, yüksek epidermal hücre yıkımı ile karakterize, tipik epidermal hiperplazi ile sonuçlanan kronik inflamatuar T hücre bağımlı bir dermatozdur. Bu çalışmanın amacı, psoriazis vulgaris patogenezinde yer alan mast hücre sayısı ile epidermal hiperproliferasyonu sağlayan p53 protein birikimi arasındaki korelasyonu göstermektir. **Gereç ve Yöntemler:** Bu çalışmada 56 psoriazis vulgaris vakasına ait punch biyopsi materyali değerlendirilmiştir. Yüzde birlik toluidin mavisi ile boyanan metakromatik granüller içeren mast hücrelerin varlığı araştırılmıştır. İlave olarak, mast hücre triptaz antikoru ile reaksiyon veren mast hücreleri incelenmiş ve epidermal keratinositlerdeki p53 boyanma oranı saptanmıştır. **Bulgular:** Psoriazis olgularında, ortalama mast hücre sayısı ile ortalama p53 boyanma oranı, sırasıyla tedavi almayan grupta (n: 20), 69.3 ± 21.5/mm^2 ve 602.3 ± 192.1, psolaren+ UVA radyasyon tedavisi alan grupta (n: 36), 57.1 ± 27.5/mm^2 ve 373.1 ± 193.6 olarak bulunmuştur. Kontrol grubunda ise, ortalama mast hücre sayısının 74.6 ± 25.9/mm^2 ve ortalama p53 boyanma oranının 176.4 ± 109.2 olduğu gözlenmiştir. Psoriazis gruplarında mast hücre sayısı ile p53 boyanma oranı arasında korelasyon bulunmamıştır. Bununla beraber, kontrol grubunda mast hücre sayısı ile p53 boyanma oranı arasında orta derecede bir korelasyon olduğu (r= 0.30 P< 0.05) bulunmuştur. **Sonuç:** Sağlıklı cilt örneklerindeki mast hücre sayısı ile p53 boyanma oranı arasında tespit edilen korelasyon, psoriazis olgularında geçerli olmadığından, bu değişkenlerin psoriazis vulgaris patogenezindeki diğer yolaklarda yer aldığı düşünülebilir.

Anahtar Kelimeler: Psoriazis; mast hücreleri; p53 proteini (325-355)

doi:10.5336/medsci.2009-15903

Psoriasis vulgaris is a common papulosquamous chronic inflammatory T-cell mediated immune relapsing dermatosis characterized by high epidermal cell turnover which results in a typical epidermal hyperplasia. It is a complex disease in which numerous abnormal findings have been reported.[1-8] However, the primary alteration is unknown. It is possible that different etiologic factors may initiate psoriasis in genetically susceptible individuals.[5,8,9] Disorders of keratinocyte proliferation, differentiation, inflammation and immune dysregulation are the major factors implicated in the pathogenesis of psoriasis vulgaris.[1-5,8]

T-cell clones obtained from psoriatic skin lesions had the selective capacity of enhancing the proliferation of keratinocytes in vitro. These T-cells are the functional link between genetic predisposition and microbial infection in psoriasis. The excessive keratinocyte proliferation reflects microbial expulsion. Mast cells are also known to have a pivotal position in bacterial defense reactions.[1,7] The close interaction of mast cells with T cells has been shown in several studies.[10]

Mast cells are mononuclear, nonphagocytic, granular and immune-effector cells found in various organs including the gastrointestinal tract, lungs and skin. Dermal infiltrate of the psoriasis consists lymphomononuclear cells and mast cells.[1,7,11-13] Recent studies have shown that mast cells may play a role in the pathogenesis of psoriasis.[2,4,5] These studies carried on by using antibody "mast cell tryptase" and "toluidin blue stain" that are specific for mast cell determination, concluded that these cells were increased in psoriasis.[1-4,14]

p53 protein is a transcription factor and an important regulator for cell cycle.[8,15-18] Proliferation of keratinocytes is restricted by apoptotic cell death to maintain a constant epidermal thickness.[1,8] Other investigations demonstrated that p53 protein was a regulatory factor in cell proliferation.[15] p53 has also been found to be positive in psoriatic lesions by immunohistochemical staining.[8,16,17] The origin of the p53 expression has been proposed to be the fast proliferation of the epidermis.[16]

The aim of this study is to investigate the relationship between two separate processes, each assumed to be a part of the pathogenesis of psoriasis vulgaris. These two processes are p53 protein accumulation in epidermal keratinocytes that provides epidermal hyperproliferation, and an increase in mast cell count of the dermis. We proposed that there was a correlation between these distinct events.

MATERIAL AND METHODS

CASES AND BIOPSIES

This study was designed as a retrospective study including punch biopsies from 56 cases with chronic type of psoriasis vulgaris diagnosed in Pathology Department of Zonguldak Karaelmas University, Faculty of Medicine, in five years. The diagnosis of chronic type of psoriasis vulgaris characterized by well-circumscribed erythematous patches with a silvery white scale was confirmed by clinical and histopathologic features. Histopathologic features consisted of parakeratosis, orthokeratosis, hyperkeratosis, regular acanthosis, elongation of the epidermal rete ridges, elongation and thinning of the suprapapillary epidermis and edema of the dermal papillae, and lymphocytic cell infiltration surrounding the capillaries. These cases were assigned randomly in two groups. Group I consisted of 20 cases (35.7%) without any treatment. Group II consisted of 36 cases (64.3%) and they had been treated by psolaren plus UVA radiation (PUVA). Healthy skin samples of 56 cases were used as the control group. Control skin samples were collected from the cases whose skin biopsies were performed for investigation of dermal tumors, especially from the margins of the wide excisions displaying no evidence of tumor.[2]

HISTOCHEMICAL AND IMMUNOHISTOCHEMICAL ANALYSES

The cross-sections of formalin-fixed and paraffin-embedded tissue samples were investigated for the presence of mast cells in which the granules were stained metachromatic with 1% toluidin blue solution (pH 4.00). To confirm the mast cell count and to reduce the technical staining problems an addi-

tional immunohistochemical staining was performed. "Mast cell tryptase" mouse monoclonal antibody (Clone AA1, LabVision, CA, USA) staining was performed at a dilution of 1:200 by streptavidin-biotin peroxidase technique to denote mast cells. The number of reactive cells were counted in 10 high power fields (HPF) (five subepidermal, three mid-dermal, two deep-dermal zones) (Leica microscope, Germany, x10 ocular, x40 objective; 1 HPF= 0.25 mm^2) [2] in order to observe the mast cell intensity in punch biopsy specimens. The mean mast cells number was calculated. p53 positivity (number of positive nuclear cells/1000) in epidermal keratinocytes was determined by using the same immunohistochemical method. The primary p53 protein mouse monoclonal antibody (Clone DO-7, Neomarkers, CA, USA) in this study was used at a dilution of 1:100.

STATISTICAL ANALYSES

The results of study were statistically analyzed using SPSS 11.0 (SPSS Inc., Chicago, Ill) statistical package program. The descriptive data were given as mean ± standard deviation. Kruskall-Wallis analysis of variance was used to determine any differences among the groups. Mann-Whitney U test was used to compare two groups. P values of <0.05 were considered as statistically significant. The correlation between mean mast cell count and mean p53 positivity was analyzed by Spearman's and Pearson's correlation tests.

RESULTS

Group I consisted of 14 (70.0%) females and six (30.0%) males with a mean age of 39.5 ± 15.4 years (range: minimum 6-maximum 63). Group II consisted of 17 (47.2%) females and 19 (52.8%) males with a mean age of 42.2 ± 13.5 years (range: minimum 12-maximum 68). Control group consisted of 31 (55.4%) female and 25 (44.6%) male cases with a mean age of 41.2 ± 14.1 years (range: minimum 6-maximum 68).

MAST CELL COUNTS

As the mean mast cells number observed either by histochemical or immunohistochemical method was too close to each other, only one calculated mean number was documented. The mean mast cells counts encountered in each group are presented in Table 1. Numerous degranulated mast cells were observed in the papillary dermis of the psoriasis groups (Figure 1). The number of the mast cells was more in the control group (Figure 2). There were significant differences between three groups, with regard to mast cell counts (P= 0.006). While no statistically significant difference was determined between control group and group I (P> 0.05); there were significant differences between control group and group II (P= 0.002), and, group I and group II (P= 0.045). The mean number of mast cells was significantly lower in group II when compared with control group (Table 1).

p53 PROPORTION

Mean p53 positivity encountered in each group is presented in Table 1. Intense p53 protein expression was detected in keratinocytes of the acanthotic psoriatic epidermis whereas p53 protein positive keratinocytes were observed only in the basal layer of the normal epidermis of the control group (Figure 3). The mean p53 proportion was significantly higher in group I and group II when compared to the control group. The mean p53 positivity was significantly lower in group II when compared to group I (Table 1). There were significant differences between three groups with regard to mean p53 positivity (P= 0.001). There were also significant differences between control group and group I, control group and group II, and, group I and group II (P= 0.001).

CORRELATION OF MAST CELL NUMBERS AND p53 PROPORTION

There was no significant correlation between mean mast cell counts and mean p53 positivity either in group I or in group II. On the contrary, a moderate correlation was found in control group regarding mean mast cells count and mean p53 positivity (r= 0.30 P< 0.05).

DISCUSSION

Psoriasis vulgaris is a common benign hyperproliferative disorder of the skin which affects approximately 2% of the population.[9,18-21] It is histopathologically characterized by parakeratosis,

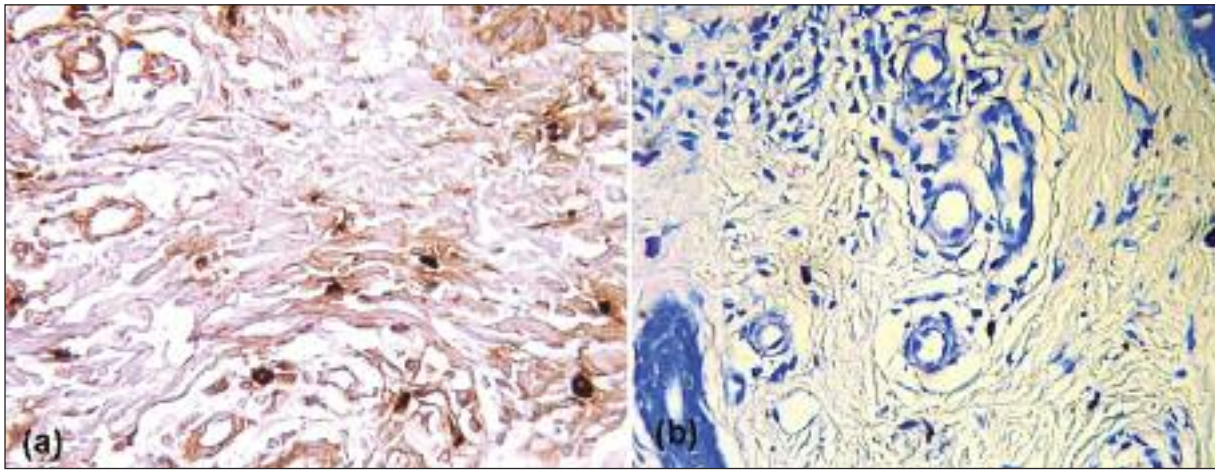

FIGURE 1: Mast cells in psoriasis vulgaris. Immunohistochemical staining of mast cell tryptase in group I (B-SA, DAB, x400) **(a)** and histochemical staining of 1% toluidine blue (pH 4.00) in group II **(b)**.

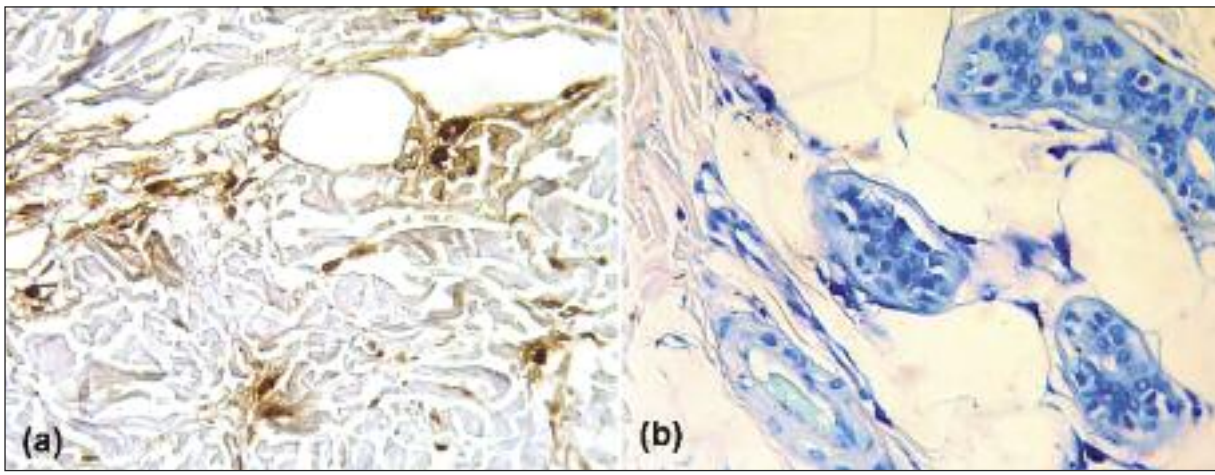

FIGURE 2: Mast cells in control group. Immunohistochemical staining of mast cell tryptase (B-SA, DAB, x400) **(a)** and histochemical staining of 1% toluidine blue (pH 4.00) (**b**).

TABLE 1: Total number of mast cells and p53 proportion in skin biopsies.

	Control group (n= 56)	Group I (n= 20)	Group II (n= 36)	p^a	p^b	p^c
Mast cells/mm^2						
Mean ± SD	74.6 ± 25.9	69.3 ± 21.5	57.1 ± 27.5	>0.05	0.002	0.045
p53 positivity						
Mean ± SD	176.4 ± 109.2	602.3 ± 192.1	373.1 ± 193.6	0.001	0.001	0.001

SD: Standard deviation.

p^a: Comparison of control group and group I.

p^b: Comparison of control group and group II.

p^c: Comparison of group I and group II.

hyperkeratosis, regular acanthosis, elongation of the epidermal rete ridges, elongation and edema of the dermal papillae, and lymphocytic cell infiltration.[11,21-23] This was also confirmed in our study. Psoriasis usually runs a chronic course although spontaneous or treatment-induced remissions may occur.[5]

Two major pathological steps exist in psoriasis vulgaris: Epidermal hyperproliferation with

abnormal differentiation, and inflammatory infiltration of epidermis and dermis.[1-4,19,22,23] Inflammation is prominent whereas hyperproliferation is less conspicuous in eruptive guttate psoriasis. Marked epidermal thickening and a less prominent inflammatory component are the main characteristics of chronic plaque-type psoriasis.[3] All of the cases enrolled into our study were chronic plaque-type psoriasis, having less inflammatory component and marked hyperproliferation.

Recently, a novel pathogenetic concept has been gaining importance which explains psoriasis as an immunologically mediated disorder and attributes the activation of T cells in the skin with an important role in psoriasis manifestation.[7] Therefore, hyperresponsiveness of psoriatic keratinocytes to growth-promoting signals from T cells was suggested to contribute to epidermal hyperplasia.[6] We designed this study to test the hypothesis about the possibility of a correlation between these two major pathological steps that take place in the pathogenesis of psoriasis vulgaris. In this study, mean mast cell counts in dermis and p53 protein accumulation in epidermal keratinocytes of psoriasis vulgaris were evaluated.

Mast cells are capable of releasing a wide range of factors that regulate the inflammatory process in skin disorders including psoriasis.[1,3,11-13,21] These indicate a potential initiator role of mast cells in the pathogenesis of psoriasis.[1,13] Mast cell-mediated vascular alteration is suggested to play a role in the development of psoriasis.[4,12,13] Mast cell numbers of involved psoriatic skin lesion are significantly increased in the upper dermis as compared to normal skin,[2-5,10-12] early lesions or Koebner phenomenon-induced lesions are also associated with increased mast cell numbers.[4,10,13] Yamamoto et al.[4] showed that mast cells were increased in number in the skin lesion of eight patients with psoriasis vulgaris complaining itching (56.3 ± 22.3), whereas the mast cell numbers displayed no increase in skin lesions of 12 patients with psoriasis vulgaris having no itching complaint (31.5 ± 10.3). Ozdamar et al.[2] found that the number of mast cells reacted positively with toluidin blue was significantly higher in psoriatic skin lesion when compared to the controls. In the present study, there was not any increase in the mean mast cell counts of psoriatic cases, thus our study was not able to confirm previous findings. In addition, control skin biopsies demonstrated significantly elevated mast cell counts when compared to psoriatic skin lesions. Degranulated mast cells were observed in the upper dermis of psoriatic skin lesions in the present study, as it was reported in the literature.[1,2,4,10,12] In addition, biopsies in our group I and group II psoriatic cases demonstrated increased mean mast cell counts (69.3 ± 21.5; 57.1 ± 27.5) when compared to the study of Yamamoto et al.[4] Skin biopsies in our control group displayed a higher mean mast cell count (74.6 ± 25.9) when compared to Jiang et al (47.5 ± 32.5).[11]

It has been shown that mast cell density increases with the onset of clinically visible lesions, and this higher density remains elevated as long as the inflammation persists. Treatment regimens for psoriasis are known to substantially reduce mast cell numbers at lesional sites as the regressed plaques.[10,11] This was also observed in our study. There was a partial reduction of mast cell numbers at the site of regressed psoriatic lesions.[3,11] Group II of our study supported this literature finding about the reduction of mean mast cell count. Smaller mast cell counts denoted in group I and II psoriasis vulgaris cases when compared to the control group suggested that these were not the early stage lesions of psoriasis vulgaris, but a chronical type of lesion that regressed with diminished inflammatory component.

Keratinocytes can express Fas, FasL, tumor necrosis factor receptor (TNFR), TNF-α and p53.[24] The expression and cellular localization of p53 protein in human epidermis and keratinocytes are poorly understood.[15-17,25] Previous studies showed that p53 protein was present at low levels in normal cells.[15-17] p53 mutations have been demostrated in sun-exposed but histologically normal-looking epidermis in characteristic compact patches of p53 immunopositive keratinocytes.[16,17,25-27] One explanation for this entity was that in psoriasis, increased keratinocyte proliferation was associated with increased proto-oncogene expression level, and the delivery of cell activation signals via certain oncogenes such as c-myc could simultaneously stimula-

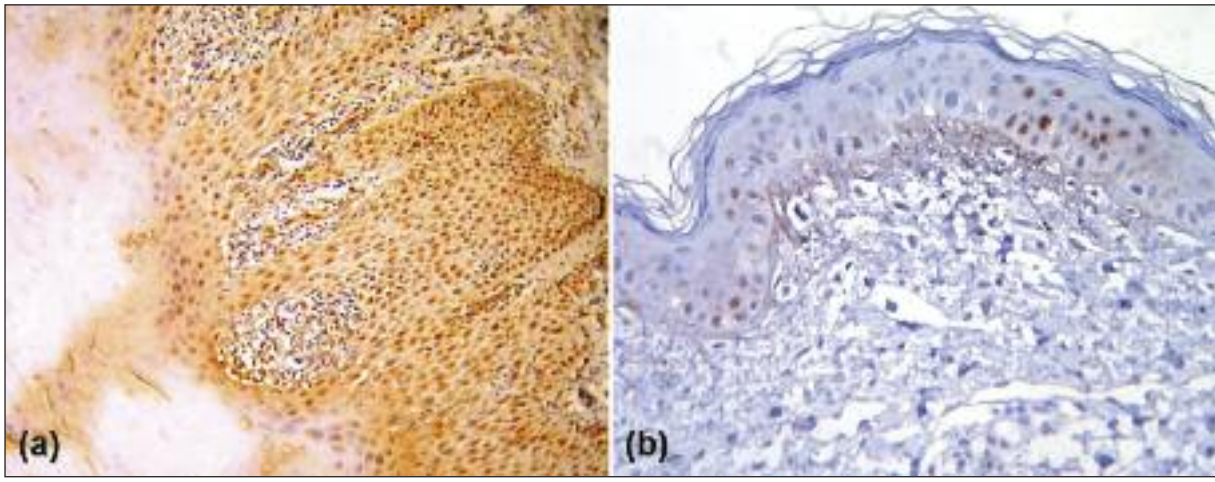

FIGURE 3: Immunohistochemical staining of p53 protein in psoriatic epidermis **(a)** and normal epidermis **(b)** (B-SA, DAB, x400).

te proliferation and apoptosis.[15,17,18,23,27] Therefore ultraviolet radiation type B (UVB)-induced apoptosis can be mediated by either the Fas or the p53 pathways.[24] Induction of apoptosis in epidermal keratinocytes appears to be the mechanism responsible for the involution of the psoriatic hyperplasia that follows PUVA.[5]

Our study reproduced the literature data about the cytoplasmic and nuclear reactivity in basal keratinocytes and expression of cytoplasmic protein throughout the epidermis in psoriasis with p53 protein. A couple of studies disclosed an increase in cellular proliferation in psoriatic lesions using p53 protein expression.[15,16,18,27] The present study is concordant with these findings. Hannuksela-Svahn et al.[16] reported that the number of p53 positive keratinocytes increased in half of the patients in psoriatic lesions and in 75% of the non-lesional skin samples after PUVA. In our study, we found significant increase in p53 immunoreactivity both in group I and group II psoriasis cases when compared to the control group. We suggest that the reduction in p53 immunoreactivity in group II when compared to that of group I may be the result of the PUVA treatment.

Psoriasis is a chronic inflammatory skin disorder that is currently not curable. Many treatment modalities exist, but the disease is often resistant to treatment. The relapses are frequent on cessation of medication. Although the cause of psoriasis is still unknown, complex interactions between altered keratinocyte proliferation and immune dysregulation are accused.[12,14,19] Both cellular and non-cellular components of the immune system have a role in the psoriatic process.[20,24]

This study was designed to disclose whether there was a correlation between mean mast cells counts and mean p53 positivity in psoriatic cases or not. When compared to the psoriatic groups, there was a noticeable increase in number of mast cells in the control group. We found increased p53 staining in all psoriatic cases and especially in group I. Although we observed a correlation between mean mast cells count and mean p53 positivity in the control group, no correlation was observed in psoriatic cases. The choice of study group among the patients with chronic-type psoriasis can be thought as the most important limitation of this study.

As the correlation observed between mean mast cell numbers and mean p53 proportion in the control group was not observed either in treated or in untreated psoriasis vulgaris groups, we can conclude that these two factors may act via different independent pathways in the pathogenesis of psoriasis vulgaris.

REFERENCES

1. Doger FK, Dikicioglu E, Ergin F, Unal E, Sendur N, Uslu M. Nature of cell kinetics in psoriatic epidermis. J Cutan Pathol 2007; 34(3):257-63.
2. Ozdamar SO, Seçkin D, Kandemir B, Turanli AY. Mast cells in psoriasis. Dermatology 1996; 192(2):190.
3. Christophers E, Mrowietz U. The inflammatory infiltrate in psoriasis. Clin Dermatol 1995; 13(2):131-5.
4. Yamamoto T, Katayama I, Nishioka K. Possible contribution of stem cell factor in psoriasis vulgaris. J Dermatol Sci 2000;24(3):171-6.
5. Weedon D. The psoriasiform reaction pattern. Skin Pathology. 2nd ed. London: Churchill Livingstone; 2002. p.75-96.
6. Prinz JC. Which T cells cause psoriasis? Clin Exp Dermatol 1999;24(4):291-5.
7. Prinz JC. Psoriasis vulgaris – a sterile antibacterial skin reaction mediated by cross-reactive T cells? An immunological view of the pathophysiology of psoriasis. Clin Exp Dermatol 2001;26(4):326-32.
8. Bülbül Başkan E, Tunalı Ş, Filiz G, Aydoğan K, Sarıcaoğlu H. [Evaluation of Ki-67, PCNA, bcl-2 and p53 protein expressionsin psoriatic lesions with immunohistochemical methods]. Turkiye Klinikleri J Dermatol 2001;11(2):68-72.
9. Solak Tekin N, Koca R, Altınyazar HC, Çınar S, Muhtar Ş, Aslaner NN. [The evaluation of the sociodemographic and clinical features of psoriasis patients in the region of Zonguldak]. Turkiye Klinikleri J Dermatol 2005;15(3):141-6.
10. Harvima IT, Nilsson G, Suttle MM, Naukkarinen A. Is there a role for mast cells in psoriasis? Arch Dermatol Res 2008;300(9):461-78.
11. Jiang WY, Chattedee AD, Raychaudhuri SP, Raychaudhuri SK, Farber EM. Mast cell density and IL-8 expression in nonlesional and lesional psoriatic skin. Int J Dermatol 2001; 40(11):699-703.
12. Ackermann L, Harvima IT. Mast cells of psoriatic and atopic dermatitis skin are positive for TNF-alpha and their degranulation is associated with expression of ICAM-1 in the epidermis. Arch Dermatol Res 1998;290(7): 353-9.
13. Apaydın R, Bahadır S.[Mast cells in dermatology]. Turkiye Klinikleri J Dermatol 1999; 9(3): 167-74.
14. Ackermann L, Harvima IT, Pelkonen J, Ritamäki-Salo V, Naukkarinen A, Harvima RJ, et al. Mast cells in psoriatic skin are strongly positive for interferon-gamma. Br J Dermatol 1999;140(4):624-33.
15. Helander SD, Peters MS, Pittelkow MR. Expression of p53 protein in benign and malignant epidermal pathologic conditions. J Am Acad Dermatol 1993;29(5 Pt 1):741-8.
16. Hannuksela-Svahn A, Pääkkö P, Autio P, Reunala T, Karvonen J, Vähäkangas K. Expression of p53 protein before and after PUVA treatment in psoriasis. Acta Derm Venereol 1999;79(3):195-9.
17. Hall PA, McKee PH, Menage HD, Dover R, Lane DP. High levels of p53 protein in UV-irradiated normal human skin. Oncogene 1993;8(1):203-7.
18. Tadini G, Cerri A, Crosti L, Cattoretti G, Berti E. P53 and oncogenes expression in psoriasis. Acta Derm Venereol Suppl (Stockh) 1989;146:33-5.
19. Griffiths CE, Voorhees JJ. Immunological mechanisms involved in psoriasis. Springer Semin Immunopathol 1992;13(3-4):441-54.
20. Baker BS, Fry L. The immunology of psoriasis. Br J Dermatol 1992;126(1):1-9.
21. Ashenagar MS, Sugihara K, Maeda A, Isogai R, Takahashi M, Aisu K, et al. The presence of tryptase-positive and bikunin-negative mast cells in psoriatic skin lesions. Arch Dermatol Res 2007;298(9):421-6.
22. Yazici AC, Tursen U, Apa DD, Ikizoglu G, Api H, Baz K, et al. The changes in expression of ICAM-3, Ki-67, PCNA, and CD31 in psoriatic lesions before and after methotrexate treatment. Arch Dermatol Res 2005;297(6):249-55.
23. Kawashima K, Doi H, Ito Y, Shibata MA, Yoshinaka R, Otsuki Y. Evaluation of cell death and proliferation in psoriatic epidermis. J Dermatol Sci 2004;35(3):207-14.
24. Rafferty TS, Beckett GJ, Walker C, Bisset YC, McKenzie RC. Selenium protects primary human keratinocytes from apoptosis induced by exposure to ultraviolet radiation. Clin Exp Dermatol 2003;28(3):294-300.
25. Campbell C, Quinn AG, Angus B, Farr PM, Rees JL. Wavelength specific patterns of p53 induction in human skin following exposure to UV radiation. Cancer Res 1993;53(12): 2697-9.
26. Swale VJ, Quinn AG. Tumour suppressor genes. Clin Exp Dermatol 2000;25(3):231-5.
27. Ekmekçi PK, Anadolu R, Erdem C. [P53 gene in dermatology]. Turkiye Klinikleri J Dermatol 2000;10(2):163-8.