AMERICAN JOURNAL OF RESEARCH IN MEDICAL SCIENCES, 2018
VOL 2, NO. 1: 34-43
10.5455/ajrms.281926

eJManager

ORIGINAL ARTICLE

Open Access

Study of keratinocyte proliferation, apoptosis and dermal inflammation in psoriatic skin lesions before and after methotrexate therapy. Which change is contributing more to clinical severity of the disease?

Amany Abdel Bary, Ahmed Abdel Bary

Department of Pathology, Faculty of Medicine, University of Alexandria, Egypt

ABSTRACT

Objectives: The pathogenesis of psoriasis involves T-cell mediated immunologic response, keratinocytes hyperproliferation, and resistance to apoptosis. Methotrexate is one of the most reliable modalities in treatment of psoriasis that target those changes. The study of those variables before and after methotrexate therapy may answer the question: which of those variables contributes more to clinical severity of the disease, and is more valuable to be targeted in psoriasis treatment.

Patients and Methods: 50 cases of psoriasis vulgaris were included, 25 patients received methotrexate therapy. Skin biopsies of psoriatic skin lesions before and after treatment were examined histologically for measurement of epidermal thickness and grading of dermal inflammation. Immunostaining for Ki-67 and p53 was done for assessment of keratinocyte proliferation and apoptosis. Results were correlated with clinical severity of the disease, assessed by PASI score (psoriatic area and severity index). Fifteen biopsies of normal skin were included as control.

Results: Dermal inflammation, Ki-67% and p53% were significantly higher in psoriatic skin lesion than in normal skin. Those changes were significantly correlated with PASI score. Multiple logistic regression models revealed that Ki-67 was the most significant variable contributing to clinical severity. One unit change in ki-67% can explain 1.2 unit changes in PASI score with 97% sensitivity, 40% specificity and 25% cut-off value. PASI score, dermal inflammation, Ki-67% and P53% were significantly reduced after methotrexate therapy. No significant difference was detected between the percent reduction in PASI score (81%) and that of Ki-67% (70%).

Conclusion: Keratinocyte proliferation was the most significant variable contributing to clinical severity of psoriasis, and it was the single parameter that showed parallel changes to PASI score after methotrexate therapy. Keratinocyte proliferation may be considered as the stimulus that induces all other pathological and clinical changes in psoriasis and should be targeted by therapy.

ARTICLE HISTORY

Received 14 November 2016
Accepted 21 January 2017
Published 5 October 2017

KEYWORDS

psoriasis; proliferation; apoptosis; inflammation; methotrexate

Introduction

Psoriasis is a common, chronic, inflammatory dermatological disorder characterized by keratinocytes hyperproliferation, abnormal differentiation and resistance to apoptosis. In addition, the presence of inflammatory cell infiltrate is one of the important histologic findings in psoriasis. A complex network of interactions has been described, between keratinocytes and infiltrating T-cells in psoriatic skin lesions [1,2].

It was earlier recognized that de novo keratinocyte hyperproliferation and abnormal epidermal differentiation were the primary causes of psoriasis, but the stimulus for such cellular multiplication

Contact Amany Abdel Bary dramany_abdelbary@yahoo.com Department of Pathology, Faculty of Medicine, University of Alexandria, Egypt.

was precisely not determined. It is now recognized, that the keratinocytes in psoriatic skin lesions are stimulated to proliferate by primed T lymphocytes [3].

Moreover, it have been demonstrated that cytokines secreted from psoriatic keratinocytes potentiate T lymphocyte activation to a greater extent than cytokines secreted from normal keratinocytes. It is also postulated that only psoriatic epidermal cells respond to activated T cell messages with hyper-proliferation, because of their specific receptors or signal-transducing mechanisms [4].

Abnormal regulation of apoptosis has also been reported in the pathogenesis of psoriasis; however several contradictory findings have been issued on the expression of P53 in psoriatic skin lesions. P53 "the guardian of the genome" is a tumor suppressor gene located on short arm of chromosome (17 p13.1). The wild type P53 negatively regulates cellular proliferation by controlling the cell cycle. P53 mutation is involved many neoplastic and non-neoplastic diseases [5].

During the last three decades, methotrexate has been used in the treatment of psoriasis with well-established record of efficacy. Even with the era of novel biological agents, methotrexate remains essential for the treatment of moderate and severe psoriasis. Despite the long history, methotrexate's mechanism of action in psoriasis remains not entirely clear. Several immunomodulatory and immunosuppressive effects have been ascribed to methotrexate using in vitro and in vivo studies [6]. Recently it is becoming more apparent that methotrexate may intercept several points in the pathways that lead to psoriasis. In addition to the anti-metabolite activity of methotrexate that inhibits keratinocyte proliferation, methotrexate may induce keratinocyte apoptosis and hinders its maturation; it also induces T-cell cytotoxicity and down regulates adhesion molecules involved in T-cell migration [7-9].

Study of the relationship between histological changes and clinical severity of psoriatic cases before and after methotrexate therapy may participate in understanding the precise stimuli evoking this disease and affecting its clinical progression.

Patients and Methods

This study included 50 newly diagnosed cases of psoriasis vulgaris. The patients were submitted to the Dermatology and Venereology Department, Faculty of Medicine, Alexandria University, Egypt, during the period between June 2014 and September 2015. Patients on treatment and recurrent cases were excluded from the study.

Clinical assessment of psoriatic patients was performed through calculation of psoriatic area and severity index (PASI score) [10].

PASI Score

The four main body areas were assessed: the head (h), the trunk (t), the upper extremities (u), and the lower extremities (I) corresponding to 10, 20, 30 and 40% of the total body area, respectively (Table 1).

Biopsy

Five mm thick incisional biopsies were taken from patients' psoriatic lesions. The biopsies were fixed in 10% neutral buffered formalin and paraffin embedded blocks were prepared. One (H&E) stained tissue section was prepared for each case and examined by light microscopy, for:

- Confirmation of the clinical diagnosis.
- Measuring the epidermal thickness using a calibrated ruler. The mean distance from the bottom of the stratum corneum to the bottom of the rete ridge was calculated from the measurement of five fields (at a magnification of x100).
- Semi-quantitative grading of the dermal lymphocytic inflammation using a three graded score: grade 1: mild, scant dermal lymphocytic infiltration, grade 2: moderate dermal lymphocytic infiltration and grade 3: severe dermal lymphocytic infiltration [11].
- Fifteen skin biopsies (from the skin of mammoplasty procedure) were included as control.

Immunohistochemical Staining

Immunostaining was performed using an avidin-biotinylated immune-peroxidase method. The primary monoclonal antibodies: anti-Ki-67 (Rabbit monoclonal antibody, clone SP6), and anti-P53 (clone D07) (thermo Scientific Lab Vision, USA) were used. The detection system was provided by Lab Vision Corporation (Neo Markers, Fremont, USA).

The deparaffinized tissue sections were rehydrated in graded alcohols. The endogenous peroxidase was blocked using 0.3% hydrogen peroxide for 20 min. For antigen retrieval, sections were microwaved in a thermoresistant container (coplin jar) containing citrate (10mM, pH 6.0). The primary antibody was then applied

Table1. The Psoriasis Area and Severity Index (PASI) is a quantitative rating score for measuring the severity of psoriasis lesions based on area coverage and plaque appearance

Plaque characteristic	Lesion score	Head	Upper limbs	Trunk	Lower limbs
Erythema	0 = none 1 = slight 2 = moderate 3 = severe 4 = very severe				
Induration					
Scaling					
Add together each of the 3 scores for each body region to give 4 separate sums(A)					
Lesion score sum (A)					
Percentage area affected	Area score	Head	Upper limbs	Trunk	Lower limbs
Area score (B): degree of involvement as a percentage for each body region affected (score each region with score between 0-6)	0 = 0% 1 = 1-9% 2 = 10-29% 3 = 30-49% 4 = 50-69% 5 = 70-89% 6 = 90-100%				
Multiply lesion score sum (A) by area score (B), for each body region, and give 4 individual subtotal (C)					
Subtotal (C)					
Multiply each of the subtotals (C) by amount of body surface area presented by that region, i.e., ×0.1 for head, ×0.2 for upper body, ×0.3 for trunk, and ×0.4 for lower limbs					
Body surface area		×0.1	×0.2	×0.3	×0.4
Totals(D)					
Add together each of the scores for each body region to give the final PASI score.					

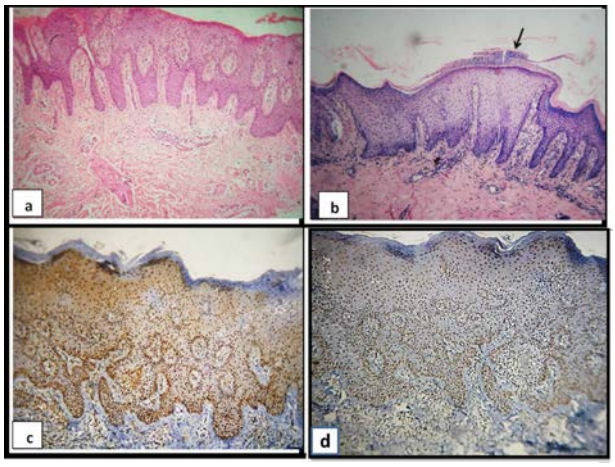

Figure 1. A case of psoriasis vulgaris showing test tube acanthosis, mild dermal inflammation and vascular proliferation (H&E x100) (a), parakeratosis and moderate dermal inflammation (H&E x100) (b), diffuse ki-67 immunostaining in keratinocytes (x100) (c) and diffuse p53 immunostaning in keratinocytes (x100) (d).

at a concentration of 1:300 for anti-Ki-67 and 1:100 for anti-P53. The reaction product was developed using diaminobenzidine tetrahydrochloride (DAB) mixture for 10 min. The DAB mixture was freshly prepared for each run (for each slide: 2 µl of DAB chromogen added to 100 µl of DAB substrate. Slides were counterstained with hematoxylin, dehydrated and mounted.

Semi-quantitative evaluation of the immunostained slides was performed by counting 1000 keratinocytes in randomly selected four consecutive epidermal fields (magnification x400). The number of positive keratinocytes was expressed as the percentage of the total number of keratinocytes. Only the nuclear staining was taken into consideration [12].

Methotrexate Treatment and Follow-up

Twenty five psoriatic patients were treated with weekly intramuscular injection of methotrexate (15mg per week). Patients were subjected to needed investigations including complete blood count, SGOT, SGPT, Urea, creatinine and hepatitis C antibodies, repeated each two weeks. Folic acid in an oral dose of 5 mg/day was given to the patients except on the day of the methotrexate injection. Patients were followed up for 2 months and monitoring of the patients was done using liver function tests, kidney function tests, and complete blood picture every 2 weeks. Exclusion criteria included patients with elevated liver enzymes and patients with mild psoriasis.

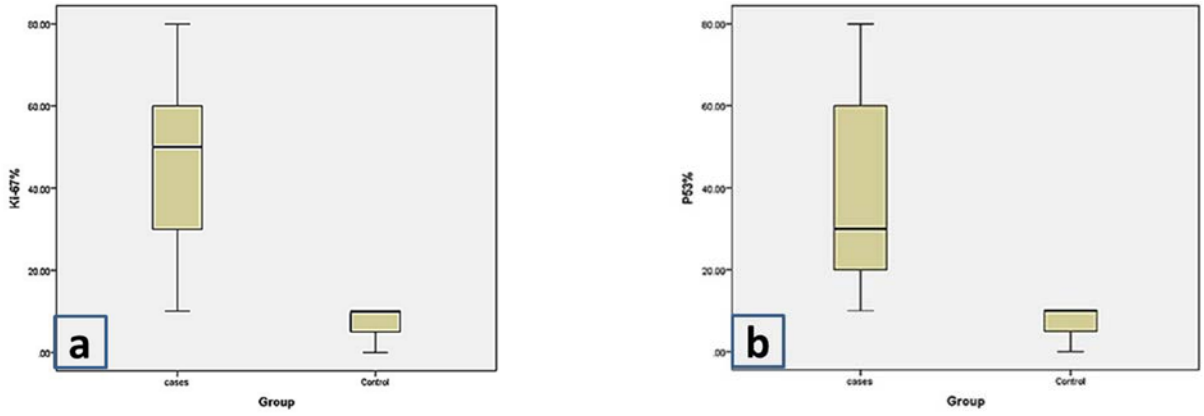

Figure 2. Two box-plot graphs showing difference between cases and control are regard ki-67% (a) and p53% (b).

Table 2. Results of the studied variables in relation to PASI score.

PASI score	Low (<10) N = 15	High (>10) N = 35
Epidermal thickness (mean)	2.2 ± 1.2	6.2 ± 4.1
Dermal inflammation grades		
Mild	8 cases (53.4 %)	3 cases (8.6%)
Moderate	5 cases (33.3 %)	20 cases (57.1%)
Severe	2 cases (13.3 %)	12 cases (34.3%)
Ki-67%		
Range	10-70%	20-80%
Median	(20%)	(60%)
p53%		
Range	10-60%	10-80%
Median	(20%)	(40%)

PASI: Psoriasis area and severity index.

Patients completed the study were subjected to re-assessment of PASI score and post-treatment biopsy was manipulated in the same way as the pre-treatment biopsy.

Statistical Analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS ver.20 Chicago, IL, USA).

Spearman rank correlation test was used for Correlation between quantitative variables.

Monte carlo significance test was used for Correlation between qualitative variables.

Multiple Logistic regression model was done using enter method for contribution to high PASI score (significant variables by univariate analysis were entered and variables causing multicolinearity problems were excluded) the model was assessed using Nagelkerk R^2 and odds ratio with 95% CI.

ROC curve analysis was used to determine sensitivity, specificity and cut-off values of Ki-6% 7 and P53% in predicting the changes of PASI score. Marginal homogeneity test was used to compare ordinal variables before and after therapy.

In all statistical tests, level of significance of .05 used, below which the results considered to be statistically significant.

Results

Results before Methotrexate Therapy

Fifty newly diagnosed cases of psoriasis vulgaris were included, the age of the patients ranged from 18 to 79 years with a mean of 55.2 ± 16.8 years. Thirty two cases were males (64%) and 18 were females (36%). PASI score ranged between 1.2 and 31.6 with a mean of 13.2 ± 7.3 and a median range of 12.7%.

Microscopic examination of the psoriatic lesions revealed epithelial hyperplasia (acanthosis), parakeratosis, and loss of the granular cell layer.

The **epidermal hyperplasia** appeared as uniform elongation of the rete ridges that are narrow towards the surface and broad at the base. The mean epidermal thickness was 4.1 ± 3.1 mms.

The papillary dermis showed dilated tortuous capillaries and perivascular lymphocytic inflammation. The inflammation was mild in 11 cases (22%), moderate in 26 cases (52%) and severe in 13 cases (26%) (Fig. 1a & b).

Ki-67% in studied cases ranged between 10 and 80 with a median range of 50%. Proliferating keratinocytes were detected, not only in basal layer but in all layers of epidermis. **P53%** in studied cases

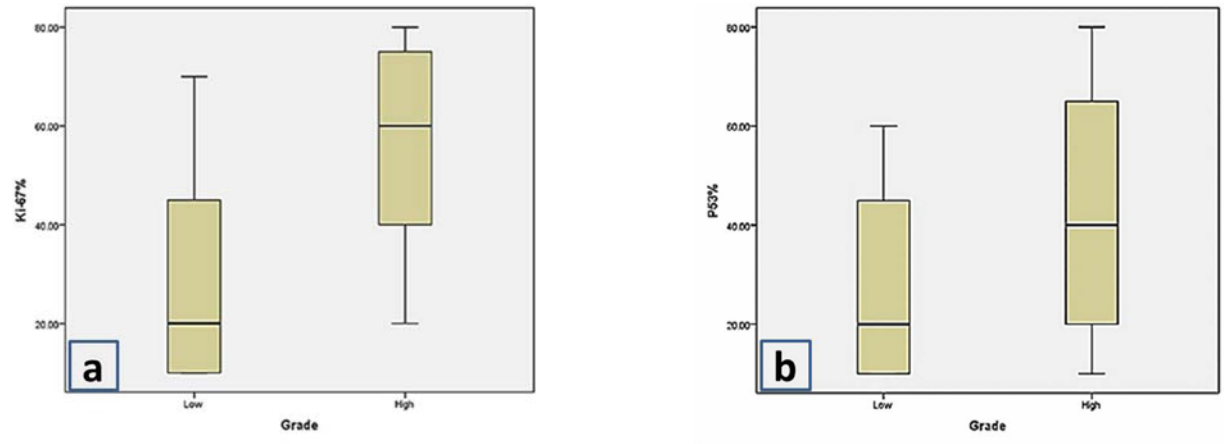

Figure 3. Two box-plot graphs showing correlation between PASI score and each of ki-67% (a) and p53% (b) in psoriatic skin lesions before treatment.

ranged between 10 and 80 with a median range of 30% (Fig. 1c & d).

In normal skin biopsies, the mean epidermal thickness was 1.5 ± 0.5. Ki-67% ranged between 0 and 10 with a median range of 10%, proliferating cells are detected in the basal layer. p53% ranged between 0 and 10 with a median range of 10%, detected mainly in supra-basal keratinocytes.

Results of Univariate Statistical Analysis

The mean epidermal thickness was significantly higher in psoriatic skin lesions than in normal skin. (p = .000 Mann-Whitney test). Ki-67% and p53% were significantly higher in cases than control; (p = .000 and .000, respectively, Mann-Whitney test) [Fig. 2].

For statistical purpose, PASI score was classified into high (>10) and low (<10). High PASI score was detected in 35 cases (70%) and low PASI score was detected in 15 cases (30%).

In cases of low PASI score, the mean epidermal thickness was 2.2 ± 1.2. Dermal inflammation was mild in 8 cases (53.4%), moderate in 5 cases (33.3%) and severe in 2 cases (13.3%). **Ki-67%** ranged between 10 and 70 with a median range of 20%. **P53%** in studied cases ranged between 10 and 60 with a median range of 20%.

In cases of high PASI score, the mean epidermal thickness was 6.2 ± 4.1. Dermal lymphocytic inflammation was mild in 3 cases (8.6%), moderate in 20 cases (57.1%) and severe in 12 cases (34.3%). **Ki-67%** ranged between 20 and 80 with a median range of 60%. **P53%** in studied cases ranged between 10 and 80 with a median range of 40% (Table 2).

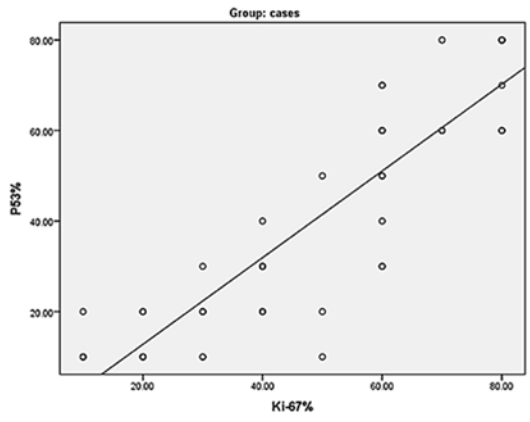

Figure 4. A scatter diagram showing the positive linear relationship between ki-67 and p53 in studied cases of psoriasis vulgaris before treatment.

Significant positive correlations were detected between PASI score and each of dermal lymphocytic inflammation, ki-67% and p53% (p = .006, .000 and .016 respectively, Mann-Whitney and Spearman's rho tests) (Fig. 3).

Significant positive correlations were detected between dermal lymphocytic inflammation and each of Ki-67% and P53% (p = .000 and .000 respectively, spearman's rho correlation.) A significant positive correlation was detected between ki-67% and p53% (p = .000, Spearman's rho correlation) (Fig. 4).

ROC curve analysis revealed that Ki-67% test can detected changes in PASI score with a sensitivity of 97% and specificity of 40%. The cut-off value of Ki 67% to differentiate cases with high PASI scores from those with low PASI score was 25%. For P53%, the sensitivity was 88.6%, the specificity was 60%, and the cut-off value was 15% (Fig. 5).

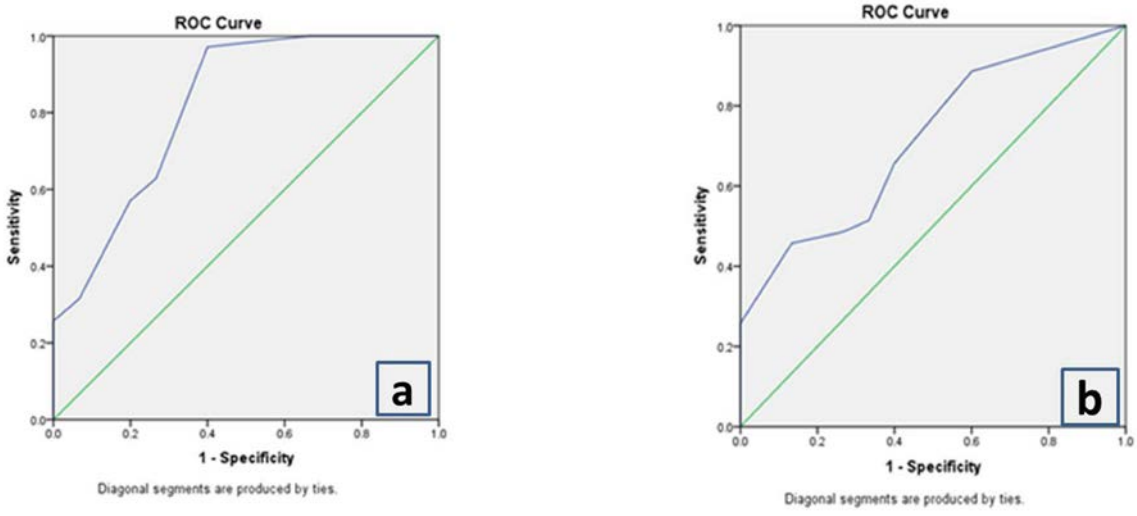

Figure 5. ROC curves showing specificity and sensitivity of Ki-67% (a) and P53% (b) for predestining changes in PASI score.

Results of Multivariate Statistical Analysis

A multiple logistic regression model was studied including PASI score and the variables proved to be significantly affecting the changes in PASI score by univvariate analysis. Those variables included dermal inflammation, Ki-67% and p53%. Two models were statistically significant, the first one was the model including Ki-67% and P53% together, (p = .000), both markers taken together can explain 53.5% of the variability in PASI score. (Nagelkerke R^2 = .535).

The second significant model was the one including Ki-67% and dermal inflammation together, (p = .000), both markers taken together can explain 42.7% of the variability in PASI score. (Nagelkerke R^2 = .427).

A simple logistic regression model testing Ki-67% was the only variable that significantly contributes in PASI score changes, while the other variables causing multicolinearity problems and were excluded.

One unit change in ki-67% can explain 1.2 unit changes in PASI score (P_{model} < 0.001, R^2 = 1.224, $P_{contribution}$ = 0.009, Odds ratio (OR) = 1.332, 95% confidence interval (1.075-1.650)].

Results after Methotrexate Therapy

From the twenty five patients treated with methotrexate, only 15 patients completed the study. Two patients developed more exacerbation of the disease, three patients stopped treatment and five patients lost follow up.

Among the 15 patients completed the study, PASI score ranged between 1.2 and 16.8 with a mean of 7.1 ± 5.2 and a median range of 7.2%.

Microscopic examination of the psoriatic lesions revealed decreased epithelial hyperplasia, (the mean epidermal thickness was 2.1 ± 1.5mms) and regained granular cell layer.

The papillary dermis showed decreased dermal inflammation and vascularity. The inflammation was mild in 8 cases (53%), moderate in 4 cases (27%) and severe in 3 cases (20%) (Fig. 6a & b).

Ki-67% in the treated cases ranged between 10 and 60% with a median range of 20%. **P53%** ranged between 10 and 60% with a median range of 20% (Fig. 6c & d).

PASI score, dermal lymphocytic infiltration, Ki67% and p53% were significantly decreased after methotrexate therapy (p = .000, .006, .000, and .014 respectively, marginal homogeneity test).

The percent reduction after methotrexate therapy was 81% for PASI score, 64% for dermal lymphocytic inflammation, 70% for Ki-67 and 64% for p53%. Both PASI score and Ki-67% showed parallel decrease with no significant difference (p = .06). While percent change in dermal lymphocytic infiltration and p53% was significantly lower than reduction in PASI score, (p = .000 and .000 respectively) (Fig. 7).

Discussion

The pathogenesis of psoriasis is very complex. It has been reported that the histopathological key

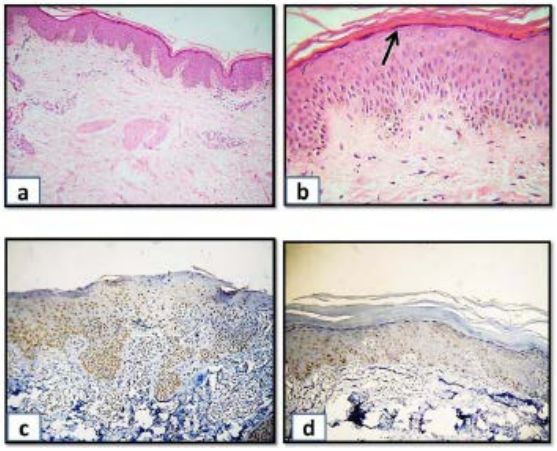

Figure 6. A case of psoriasis vulgaris after methotrexate therapy showing decreased epidermal hyperplasia, dermal inflammation and vascular proliferation (H&E x100) (a) retained granular layer (arrow) (H&E x400), (b) ki-67 immunostaining (x100) (c) and p53 immunostaining (x100) (d).

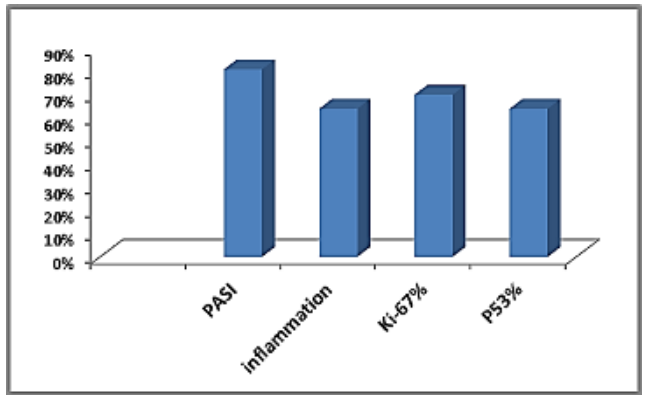

Figure 7. The percent reduction in PASI score, dermal lymphocytic infiltration, Ki-67% and P53% after methotrexate therapy.

phenomena in psoriatic plaque are accumulation of T-cell subsets that secrete a complex network of cytokines. Those cytokines are responsible for keratinocyte hyperproliferation, premature keratinization and disturbed apoptosis. At the molecular level, it was postulated that psoriatic keratinocytes have specific receptors and signaling transducing mechanisms, through which it respond to T-cell stimulation [13-15]. Psoriatic keratinocytes in turn, potentiate T-cell activation more than normal keratinocytes [4].

In a trial to search for the primary stimulus initiating the pathogenesis of this disease and affecting its clinical progression, the present work studied the most characteristic features of psoriasis, naming: keratinocyte proliferation, apoptosis and dermal lymphocytic inflammation in relation to changes in PASI score, before and after methotrexate therapy. The latter is considered as one of the most effective modalities in treatment of psoriasis. Its action involves changes in T-cell immunologic response, keratinocyte proliferation and apoptosis.

The analysis of the Ki-67 index using immunohistochemical technique is one of the most common indices of cell proliferation. It allows for quick and reliable evaluation of the growth fraction of the studied cell population (16). In our study, the median range of Ki-67% in psoriatic cases was 50%. We reported significant higher Ki-67 expression in psoriatic skin lesions that in normal skin, in accord with most of the reviewed literatures, but they reported different figures of expression [12,17-19]. Adi et al, reported 10.8% Ki-67 expression in psoriatic keratinocytes [12]. Jesionek-kupnicka reported up to 30% median range [19], while Hannuksela-Svahn reported a median range of 16.6% [20]. The epidermal thickness was significantly higher in psoriatic skin lesions than in normal skin, similar results were reported by Tursen et al [21] and Jang et al [22]. In our study and many other reported studies, proliferating keratinocytes were detected in supra-basal cells, while in healthy skin, it was detected in basal keratinocytes [23-26].

Abnormal apoptotic activity is another important ultra-structural features detected in many skin lesions. Several immunohistochemical markers have been used for assessment of apoptosis, of which p53 is the most popular [5]. Although p53 immunoreactivity has also been found in several inflammatory skin diseases, the first publication to discuss p53 and psoriasis was in 1989, when Tadini et al. reported that anti P53 polyclonal antibody was positive in about 20% of the cells in the basal and suprabasal layers of psoriatic skin [27]. More recently, Baran et al reported median P53% expression of 20% in psoriatic lesions [5]. Other studies reported much smaller figures [5,27]. In our study the median p53% expression in psoriatic lesions was 40%. The high values of Ki-67% and P53% in our study may be attributed to the fact that 70% of the cases included in our study were of high clinical severity which was significantly associated with higher Ki-67 and P53 expression. In addition, the wide variation in the results obtained by different works can be attributed to the different

immunohistochemical antibodies that recognize different epitopes and the difference in the sensitivity of the kits used [5].

In our study P53% was significantly higher in cases than in control. Up-regulation of p53 in psoriasis has been demonstrated in many studies. (5, 12, 28, 29, 30). Increased expression of p53 protein in psoriatic lesions has been explained in two different ways, some authors reported that accumulation of P53 is considered as a physiological reaction, and is thought to be involved in the inhibition of increased cell proliferation and in the repair of the possible DNA errors occurring in the rapidly proliferating tissue [19]. (29) While other authors reported that the normal wild type P53 protein has a very short half-life, thus, its concentration is generally below immunohistochemical detection level. Conversely mutant P53 protein has longer half-life, thus could be detected. It was hypothesized that detectable levels of P53 in tissue implied a mutant type. (5) While Baran et al reposted that D07 monoclonal antibody (which is the one used in the present study) detects both the wild-type and the mutated form of the p53 protein and it is possible that other factors, not only mutation, may account for the accumulation of this protein [29].

A significant positive correlation between Ki-67% and P53% was reported in our study, in accord to what has been reported by many authors [2,5,30], and this suggests that P53 accumulation was enhanced by cell proliferation [5,19]. Furthermore, both markers were significantly related to dermal lymphocytic infiltration, and this is in accord with most the studies that focused on psoriasis pathogenesis, they reported a complex network of interaction between infiltrating T-lymphocytes and keratinocytes, and that T-cells may be considered as the main regulators of keratinocytes proliferation, differentiation and apoptosis [4,13,15,32].

In our study, a univariate analysis was used to correlate the histologic parameters of psoriasis and PASI score. The latter is a known clinical indicator of the disease severity, used with many authors [5,19,29,32]. Significant positive correlations were detected between PASI score and each of dermal lymphocytic infiltration, Ki-67%, and p53%. ROC curve analysis revealed that Ki-67% assessment can detect changes in PASI score with a sensitivity of 97%, specificity of 40% and cut off value of 25%. While, P53%, can detect changes in PASI score with a sensitivity of 88.6%, specificity of 60% and cut-off value of 15%. Some authors point out the correlation between psoriatic activity, measured with the PASI index, and epidermal proliferative and apoptotic activities [5,19,32]. The significant correlation confirms that increased proliferation and apoptosis of epidermal cells results in more severe psoriasis.

But when a multilogestic regression analysis was used Ki-67% was the sole parameter that significantly contributes to changes in PASI score. One unit change in ki-67% can explain 1.2 unit changes in PASI score. This may give us a clue that epidermal cell proliferation is considered as the denominator for clinical and pathologic changes occurring in psoriasis.

For confirmation of our results, histologic changes of psoriasis were studied after application of one of the common modalities of psoriasis treatment, i.e methotrexate. In our study PASI score, dermal lymphocytic infiltration, ki-67% and p53% were significantly reduced after methotrexate therapy. Those relations are very logic, as it is known that methotrexate reduces keratinocyte hyperproliferation and influences various inflammatory mediators and apoptosis [6,8,9,19]. Many authors reported significant reduction in epidermal hyperplasia, inflammatory infiltrate and Ki-67% after methotrexate therapy [11,17,18,33,34].

In our study, the percent reduction in PASI score after methotrexate therapy was parallel to that of ki-67% reduction. While the reduction in P53% and dermal lymphocytic infiltration was significantly lagging after the reduction in PASI score.

The combined study of histologic, immunohistochemical and clinical changes in psoriasis performed in this work can give an idea about the disease pathogenesis and clinical progression. This approach revealed that keratinocyte proliferation evaluated by Ki-67 expression was the most significant variable contributing to changes in clinical severity both initially and after methotrexate therapy. However, further molecular studies are still needed to confirm this assumption.

Conflict of interest

No conflicts of interest.

References

[1] Kvist PH, Hagberg LO, Kemp VK, Ropke MA. Comparison of the effects of vitamin D products in a psoriasis plaque test and a murine psoriasis xenograft model. J Transl Med. 2009; 7:107.

[2] Kerkhof P. Textbook of psoriasis. 2nd edition, Blackwell Publishing Ltd., Oxford, pp 83–109, 2003.
[3] Braun-Falco O, Plewig G, Wolff HH, Burgdorf WHC. Dermatology, 2nd edition, Springer-Verlag, Berlin, pp 585–610, 2000.
[4] Das RP, Jain AK, Ramesh V. Current concepts in the pathogenesis of psoriasis. Indian J Dermatol 2009:54(1):7-12.
[5] EL-Adel R; Abdel Hameed M, El-Shaer M, Imam A, Abdel Hafez N. Immunohistochemical Study of Protein P53 in Egyptian Psoriasis. Report and Opinion 2011; 3(1):65-84.
[6] Saleh A, Mohn'd Abuhilal, Cheung B. Methotrexate in Psoriasis: From A to Z. J Turk Acad Dermatol 2010; 4 (1):1-13.
[7] Yamasaki E, Soma Y, Kawa Y, Mizoguchi M. Methotrexate inhibits proliferation and regulation of the expression of intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 by cultured human umbilical vein endothelial cells. Br J Dermatol 2003; 149:30-8.
[8] Phillips DC, Woollard KJ, Griffiths HR. The anti-inflammatory actions of methotrexate are critically dependent upon the production of reactive oxygen species. Br J Pharmacol 2003; 138:501-11.
[9] Herman S, Zurgil N, Deutsch M. Low dose methotrexate induces apoptosis with reactive oxygen species involvement in T lymphocytic cell lines to a greater extent than in monocytic lines. Inflamm Res 2005; 54:273-80.
[10] Psoriatic areas and severity index worksheet. Available from: URL: http://www.bad.org.uk/html.
[11] Yazici AC, Tursen U, Apa DD, Ikizoglu G, Api H, Baz K, Tasdelen B. The changes in expression of ICAM-3, KI67, PCNA and Cd31 in psoriatic lesions before and after methotrexate treatment. Arch Dermatol Res 2005; 297:249-55.
[12] Adi E, Gülekon A, Dursun A, Gürer MA. The effects of calcipotriol and methylprednisolone aseponate on bcl-2, p53 and ki-67 expression in psoriasis. JEADV 2006; 20:527–33.
[13] Gambichler T, Boms S, Hochdorfer B, Altmeyer P, Kreuter A. Immunohistology of amicrobial pustulosis of the folds. Clin Exp Dermatol 2007; 32:155–8.
[14] Gambichler T, Tigges C, Scola N, Weber J, Skrygan M, Bechara FG, Altmeyer P, Kreuter A. Etanercept plus narrowband ultraviolet B phototherapy of psoriasis is more effective than etanercept monotherapy at 6 weeks. Br Ass Dermatol 2011;164: 1383–86. 15. Mehlis SL, Gordon KB. The immunology of psoriasis and biologic immunotherapy. J Am Acad Dermatol 2003; 49: 44-50.
[15] Whitfield ML, George LK, Grant GD, Perou CM. Common markers of proliferation. Nat Rev Cancer 2006; 6: 99-106.
[16] Miracco C, Pellegrino M, Flori ML , Vatti R, Materno M, Andreassi L. Cyclin D1, B and A expression and cell turnover in psoriatic skin lesions before and after cyclosporin treatment. Br J Dermatol 2000; 143:950-56.
[17] Sigmundsdottir H, Johnston A, Gudjonsson JE, Bjarnason B, Valdimarsson H. Methotrexate markedly reduces the expression of vascular E-selectin, cutaneous lymphocyte-associated antigen and the numbers of mononuclear leucocytes in psoriatic skin. Exp Dermatol 2004; 13:426-34.
[18] Jesionek-Kupnicka D, Chomiczewska-Skora D, Rotszten D. Influence of phototherapy in psoriasis on Ki-67 antigen expression: a preliminary study. Pol J Pathol 2013; 2:96-103.
[19] Hanuksela-svahn A, Paakkoe P, Autio P, Reunala T, Karvonen J, Vahakangas J. Expression of p53 Protein Before and After PUVA Treatment in Psoriasis Acta Derm Venereol (Stockh) 1999; 79:195:99.
[20] Tursen B, Kara T, Tursen U, Apa DD, Gubur O. The changes in expression of Ki-67, and CD31 in psoriatic lesions before and after etanercept treatment. Hong Kong J. Dermatol. Venereol 2013; 21:5-13.
[21] Jang H, Oh C, Jo J, Kim Y, Kwon K. Detection of Telomerase Activity in Psoriasis Lesional Skin and Correlation with Ki-67 Expression and Suppression by Retinoic AcidJ Korean Med Sci 2001; 16:623-9.
[22] Körver JE, van Duijnhoven MW, Pasch MC, et al. Assessment of epidermal subpopulations and proliferation in healthy skin, symptomless and lesional skin of spreading psoriasis. Br J Dermatol 2006; 155:688-94.
[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; 35:207-14.
[24] Doger FK, Dikicioglu E, Ergin F, Shibata MA, Yoshinaka R, Otsuki Y. Nature of cell kinetics in psoriatic epidermis. J Cutan Pathol 2007; 34:257-63.
[25] Henno A, Blacher S, Lambert C, Colige A, Seidel L, Noël A, Lapière C, de la Brassinne M, Nusgens BV. Altered expression of angiogenesis and lymphangiogenesismarkers in the uninvolved skin of plaque-type psoriasis. Br J Dermatol 2009; 160:581-90.
[26] Tadini G, Cerri A, Crosti L, Cattoretti G, Berti E. P53 and oncogene expression in psoriasis. Acta Derm Venereol (Stockh); 1989; 146:33–5.
[27] Laporte M, Galand P, Fokan D, de Graef C, Heenen M. Apoptosis in established and healing psoriasis. Dernlatology 2000, 200:314-16.
[28] El-Domyati M, Barakat M, Abdel-Razek R. Expression of Apoptosis Regulating Proteins, P53 and Bcl-2, in Psoriasis. J Egypt worn Dermal01 Soc, 2006;7(1):46-52.
[29] El- Batawi MM, Shahin Z, Ramadan S, El – Ramly AZ, El- Mahgoub D, El- Komy M H and Saba S Expression of Bcl2 and P53 in psoriasis. Egypt J Dermatol and Androl; 2002; 22(1):13–7.

[30] Van Lingen RG, Ko¨rver JE, van de Kerkhof PC, Berends MA, van Rens DW, Langewouters AM, Boezeman JB, Seyger MM, de Jong EM. Relevance of compartmentalization of T-cell subsets for clinical improvement in psoriasis: effect of immune-targeted antipsoriatic therapies. Br J Dermatol 2008; 159:91–6.

[31] Chen GS, Wu TM, Yang SA, Yu HS. Quantitative assessments of physiological and biological parameters in psoriatic lesions and its correlations to the clinical severity of psoriasis. Kaohsiung J Med Sci 2001; 17:408-18.

[32] Gambichler T, Tigges C, Scola N, Weber J, Skrygan M, Bechara FG, Altmeyer p, Kreuter A. Etanercept plus narrowband ultraviolet B phototherapy of psoriasis is more effective than etanercept monotherapy at 6 weeks. Br Ass Dermatol 2011;164, 1383–86.

[33] Elango T, Thirupathi A, Subramanian S, Dayalan H, Gnanaraj P. Methotrexate normalized keratinocyte activation cycle by overturning abnormal keratins as well as deregulated inflammatory mediators in psoriatic patients. Clinica Chimica Acta 2015; 451:329–37.