

Effect of Methotrexate Therapy on p53 and Bcl2 Expression in Patients with Psoriasis: A Prospective Hospital-Based Cohort Study

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Abstract

Background: Psoriasis is a chronic, relapsing, inflammatory, and hyperproliferative skin disease. The growth of keratinocytes is regulated by a delicate balance between molecules controlling cell survival (such as Bcl2) and cell death (such as p53). The study was conducted to observe the clinical and histopathological effect of Methotrexate on psoriatic lesions. **Materials & Methods:** The immunohistochemical expression of pro-apoptotic (p53) and anti-apoptotic (Bcl2) proteins was correlated with histomorphological changes (epidermal thickness, Munro microabscess, granular layer, lymphocytic infiltrate, blood vessels) in the psoriatic skin. **Results:** A total of 35 cases of psoriasis were studied. Male predominance was seen and trunk was the initial site of involvement in 40% cases. There was a significant reduction in PASI (Psoriasis Area Severity Index) score, grade 3 cases of psoriasis and mean expression of p53 in all the histomorphological parameters in the subsequent 2 and 6 weeks of methotrexate therapy. The expression of p53 in all parameters and Bcl2 in the lymphocytes is significantly higher ($p < 0.05$) in psoriatic skin as compared to the normal skin (control group). **Conclusion:** Low dose of methotrexate (0.3-0.5 mg/kg) contribute to reduction in epidermal thickness and thereby remain an effective therapy for psoriasis.

Keywords: Hyperproliferative; Apoptosis; Biopsy; Post treatment

Introduction

Psoriasis is a primary inflammatory disorder induced and sustained by skin infiltrating lymphocytes with a secondary striking proliferation of keratinocytes and epidermal hyperplasia. The word “psoriasis” comes from Greek word meaning “the state of having the itch”.^[1] It affects about 2% of the world population with a bimodal age distribution (<30 years and > 50 years).^[2] The disease occurs equally in both sexes; Indian males however are affected twice as frequently as females and the prevalence in the country varies from 0.44 to 2.8%.^[3]

Psoriasis, a hyperproliferative keratinocytic disease, demonstrates a disturbance in equilibrium between the molecules controlling cell survival (such as Bcl2) and cell death (such as p53).^[4] Although there is no cure for psoriasis but several treatments can minimize the disease and some can also induce remission of months to years. Treatment modalities evaluated and approved for the same are methotrexate, cyclosporine, acitretin, infliximab, itolizumab, erlotinib, Narrow band-Ultraviolet B (NB-UVB) phototherapy and PUVA (Psoralen plus Ultraviolet A) phototherapy etc. either alone or in combination.^[5]

Methotrexate is an economical and effective antipsoriatic agent. It exerts an anti-mitotic action on psoriatic skin by competitively inhibiting dihydrofolate reductase and hence DNA synthesis.^[6]

It also prevents cell entry into the S phase and causes killing of further cells.^[7]

Literature on the effect of methotrexate therapy on the histological features in psoriatic lesions is limited. We undertook the present study to evaluate the impact of methotrexate on the expression of pro-apoptotic (p53) and anti-apoptotic (Bcl2) proteins and correlate it with the histomorphological features (epidermal thickness, Munro microabscess, lymphocytic infiltrate etc.) in psoriatic lesions.

Methods

The prospective study was conducted on a cohort of patients with psoriasis attending a tertiary referral center of North India over a period of 12 months. Institutional ethical clearance was obtained and the patients were included following a written informed

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consent. Histologically proven psoriasis and treatment naïve patients of all ages (defined as not having received any topical or systemic therapy for psoriasis in the form of methotrexate/ retinoids/ phototherapy/ other immuno-suppressive agents in the last 6 months) were included and the pretreatment status was designated as day 0.

All included patients were administered methotrexate (0.3-0.5 mg/kg of body weight/week, depending on disease severity) and biopsy was taken from the same site subsequently on the 14th day and at the end of 6th week of starting the therapy. Harris Hematoxylin and Eosin stain (H&E) was used and histopathological characteristics viz. Munro microabscesses, spongiform pustules, epidermal hyperplasia, parakeratosis, abnormal granular layer, dilated blood vessels and lymphocytic infiltrate in dermis were studied and graded. Each characteristic was graded as 0-3 (nil to marked) using a visual analog scale.

Monoclonal mouse antibodies for p53 (clone-DO7) and Bcl2 (clone-Bcl2/100) [manufactured by Bio SB] were used for immunohistochemistry and scoring was done as per the system devised by Liang et al. [8] Localization of immunohistochemical staining was categorized as epidermal, basal cell and lymphocyte staining. Basal cell staining was evaluated separately from epidermal cell staining because of the differences in the kinetics of the epidermal cells.

A group of age and sex matched controls (n=35) was also studied for p53 and Bcl2 expression in non-psoriatic skin lesions with an intention to validate the importance of these proteins in psoriasis. The Psoriasis Area and Severity Index (PASI) score was used as a measure of the severity of psoriatic lesions based on area coverage and plaque appearance. [9] This score was recorded in every visit prior to biopsy.

Statistical analysis

Statistical analysis was performed using the commercially available SPSS version 20. Qualitative data (munro microabscess,

epidermal thickness, p53 and Bcl2 expression score etc.) was expressed as frequency and/or percentage. Quantitative data (age, PASI score) was represented in the form of mean \pm SD. Paired t test was used to compare the mean of PASI score before and after methotrexate therapy. Wilcoxon signed rank test was used to compare the mean histomorphological features and immunostain expression score before and after therapy. Friedman test was done to compare the sequential change in histomorphological features and immunostain expression score from 0 week to 2 weeks and 6 weeks of therapy. Mann-Whitney U test was used to compare the immunostain expression score in normal epidermis and psoriatic epidermis. A p value < 0.05 was considered as significant.

Results

Of the 35 cases of psoriasis studied, 12 (34.3%) were in the 3rd to 4th decade (range 18-65 years). The mean age at diagnosis was 37.1 years and the male to female ratio was 3.35:1. All the patients presented with erythema and scaling while 94.3% (n=33) complained of itching additionally. Trunk was the most common site of involvement (n=14, 40%) followed by scalp and lower limb in equal number of cases (n=8, 22.9%); extra-cutaneous involvement was seen in 16 (45.7%) patients. Cold weather in winters aggravated the symptoms in 29 cases (82.9%). The histomorphological features studied showed a serial and significant reduction from the baseline [Table 1]. The reduction was progressive at 2 and 6 weeks of methotrexate therapy. Likewise, the PASI score showed a significant reduction from the baseline that was maintained at 2 and 6 weeks (18.25 ± 8.84 vs. 10.11 ± 5.75 vs. 5.06 ± 3.71 ; $p < 0.05$). However, complete clearance of PASI was not observed in any patient during the study period. The reduction was seen even in patients with grade 3 psoriasis; grade 0 psoriasis increased after methotrexate therapy.

The expression of Bcl2 in the lymphocytes was significantly higher ($p < 0.05$) as was the p53 expression in all cell types (i.e. epidermal, basal cells and lymphocytes) in psoriatic skin as

Table 1: Comparison of value of significance (p values) of different histomorphological features at 0 weeks, 2 weeks and 6 weeks of methotrexate therapy.

Histomorphological features	0 weeks (Mean \pm SD)	2 weeks (Mean \pm SD)	6 weeks (Mean \pm SD)	P' value between 0 weeks and 2 weeks	P' value between 0 weeks and 6 weeks	P° value
Munro microabscess	2.29 \pm 0.75	1.11 \pm 0.63	0.77 \pm 0.88	<0.001	<0.001	<0.001
Spongiform pustules	1.89 \pm 0.72	0.94 \pm 0.59	0.60 \pm 0.81	<0.001	<0.001	<0.001
Epidermal hyperplasia	2.66 \pm 0.48	1.91 \pm 0.56	1.49 \pm 0.85	<0.001	<0.001	<0.001
Parakeratosis	2.40 \pm 0.65	1.26 \pm 0.66	0.94 \pm 0.91	<0.001	<0.001	<0.001
Abnormal granular layer	2.17 \pm 0.82	1.31 \pm 0.63	0.97 \pm 0.82	<0.001	<0.001	<0.001
Dilated blood vessels	1.57 \pm 0.61	1.23 \pm 0.43	0.89 \pm 0.58	0.007	<0.001	<0.001
Lymphocytic infiltrate	2.03 \pm 0.66	1.46 \pm 0.56	1.31 \pm 0.53	<0.001	<0.001	<0.001

P' value by Wilcoxon signed rank test; P° value by Friedman test

Table 2: p53 expression in psoriatic skin at 0 weeks, 2 weeks and 6 weeks of methotrexate therapy.

p53 expression	Control	0 weeks (Mean \pm SD)	2 weeks (Mean \pm SD)	6 weeks (Mean \pm SD)	P value between control and psoriatic patients at 0 weeks	P' value between 0 weeks and 2 weeks	P' value between 2 weeks and 6 weeks	P° value
Epidermal cells	1.4 \pm 0.84	3.26 \pm 0.74	2.74 \pm 0.66	2.40 \pm 0.74	<0.001	0.006	<0.001	<0.001
Basal cells	2.4 \pm 0.70	4.14 \pm 0.88	3.74 \pm 0.98	3.17 \pm 1.01	<0.001	0.032	<0.001	<0.001
Lymphocytes	2.3 \pm 0.68	3.31 \pm 1.08	3.14 \pm 0.94	2.46 \pm 0.66	0.006	0.450	0.001	<0.001

P value by Mann-Whitney U test (2-tailed value); P' value by Wilcoxon signed rank test; P° value by Friedman test

compared to the non-psoriatic control group; Bcl2 expression in epidermal and basal cells was comparable in the two groups [Tables 2 and 3] [Figures 1 - 7].

Discussion

The expression of the pro-apoptotic p53 protein was higher while that of the anti-apoptotic Bcl2 protein was lower in psoriatic lesions as compared to non-psoriatic lesions. Methotrexate had a positive effect on the histomorphological features of psoriatic lesions. The effect was pronounced and sustained on p53 over a period of 6 weeks.

More than two-third of our patients were between 31-50 years; however, Okhandiar et al reported the highest incidence in the second decade of life. The apparent reason for this was the large number of subjects (n=3573).^[10] Likewise, the high male: female ratio in the present study in comparison to 2.46:1, 2.40:1 and 2.03:1 reported in other studies may be attributable to the variable sample sizes.^[9,11,12] The symptoms of scaling and itching are concordant with the previous study by Prignano et al.^[13]

Trunk, an uncommon site for psoriatic lesions was observed in 40% of our subjects. The reduction in the mean PASI score was similar to that observed in the studies by Yazici et al and

Table 3: Bcl2 expression in psoriatic skin at 0 weeks, 2 weeks and 6 weeks of methotrexate therapy.

Bcl2 expression	Control	0 weeks (Mean ± SD)	2 weeks (Mean ± SD)	6 weeks (Mean ± SD)	P value between control and psoriatic patients at 0 weeks	P` value between 0 weeks and 2 weeks	P` value between 2 weeks and 6 weeks	P° value
Epidermal cells	2.60±0.70	3.03 ± 0.86	3.06 ± 1.0	3.00 ± 0.87	0.182	0.966	0.925	0.972
Basal cells	3.70±0.68	4.00 ± 0.80	4.34 ± 1.08	3.80 ± 1.02	0.158	0.090	0.327	0.077
Lymphocytes	3.67±0.70	4.43 ± 0.85	4.34 ± 1.37	4.26 ± 1.27	0.005	0.989	0.437	0.918

P-value by Mann- Whitney U-test (2-tailed value); P` value by Wilcoxon signed rank test; P° value by Friedman test

Table 4: Comparison of previous studies using various therapeutic modalities for psoriasis with present study using methotrexate.

Parameters		Adisen et al. ^[19] using calcipotriol and methylprednisolone (2005)	Gomez et al. ^[20] using Infliximab (2009)	Domyati et al. ^[18] using PUVA therapy (2012)	El Gayyar et al. ^[21] using aloe vera and coal tar mixture (2012)	Present study using methotrexate (2017)
	Epidermal thickness	-	↓	significant ↓ (p<0.001)	-	significant ↓(p<0.001)
	Munro microabscess	-	↓	-	-	significant ↓(p<0.001)
	Spongiform pustules	-	↓	-	-	significant ↓(p<0.001)
	Dermal inflammatory infiltrate	-	↓	-	-	significant↓ (p<0.001)
P53 expression	Epidermal cells	significant ↓ with both (p<0.05)	significant ↓ (p<0.05)	significant ↑ (p<0.001)	-	significant↓ (p<0.001)
	Basal cells	-	-	-	-	significant↓(p<0.001)
	Lymphocytes	-	-	No significant difference	-	significant ↓(p<0.001)
Bcl2 expression	Epidermal cells	significant ↑ with both (p<0.05)	negative	significant↑ (p<0.001)	significant↓ (p<0.01)	Insignificant (p=0.972)
	Basal cells	-	-	-	v	Insignificant (p=0.077)
	Lymphocytes	-	-	Significant ↓ (p=0.01)	↓but insignificant (p>0.05)	Insignificant (p=0.918)

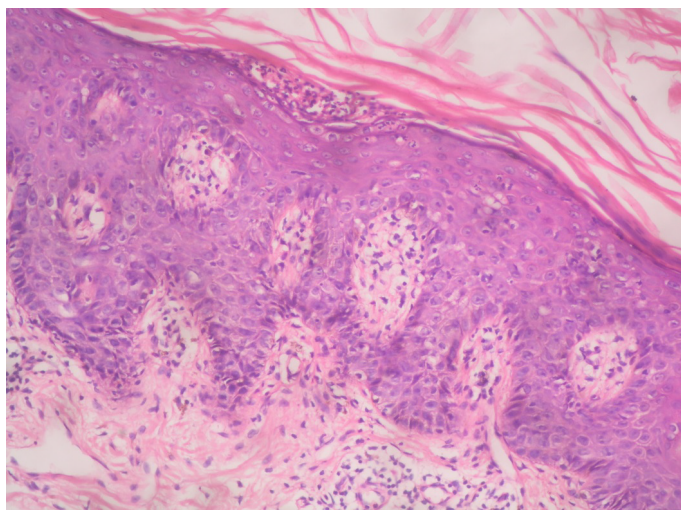


Figure 1: Skin biopsy showing reduction of munro micro abscess and reduction in epidermal hyperplasia after 2 weeks of methotrexate therapy (H&E 200X).

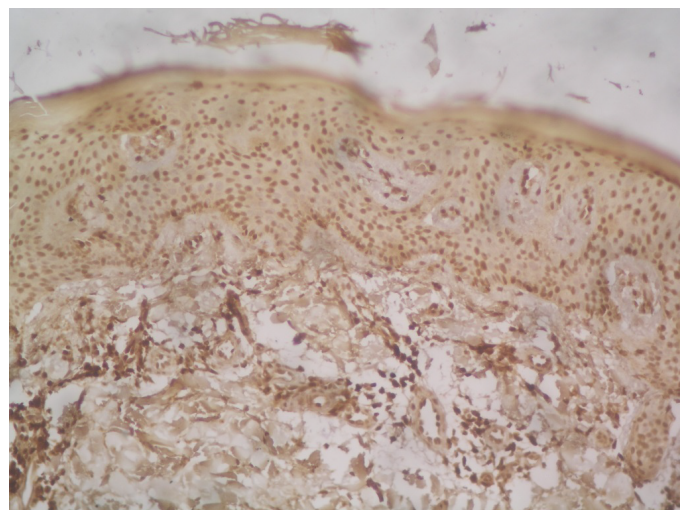


Figure 2: Skin biopsy showing moderate to intense epidermal and basal cell positivity for p53 immunostain at 0 weeks of methotrexate therapy (200X).

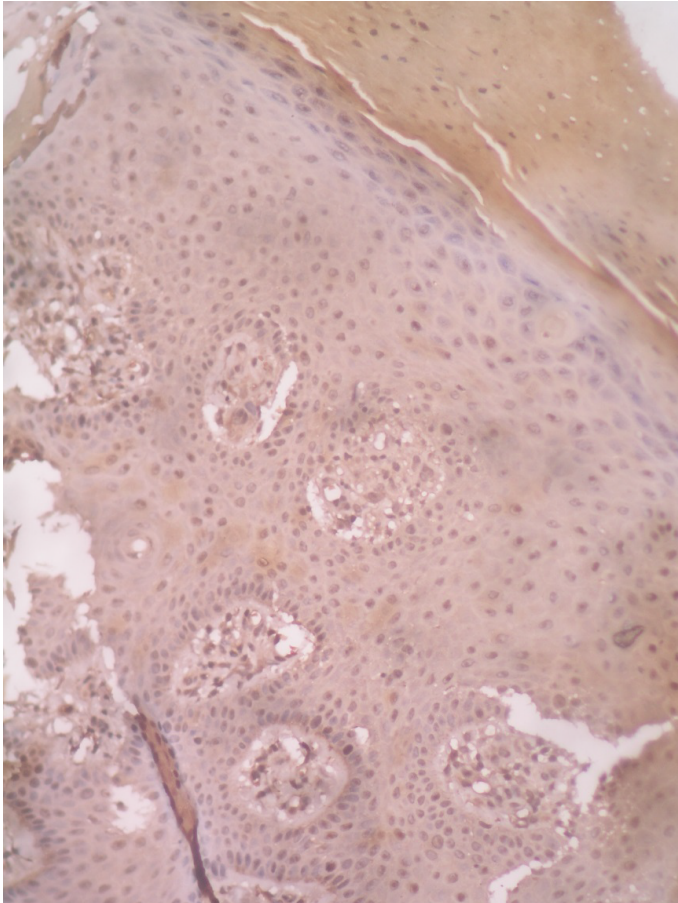


Figure 3: Skin biopsy showing mild to moderate epidermal and basal cell positivity and reduced lymphocyte positivity for p53 immunostain at 2 weeks of methotrexate therapy (200X).

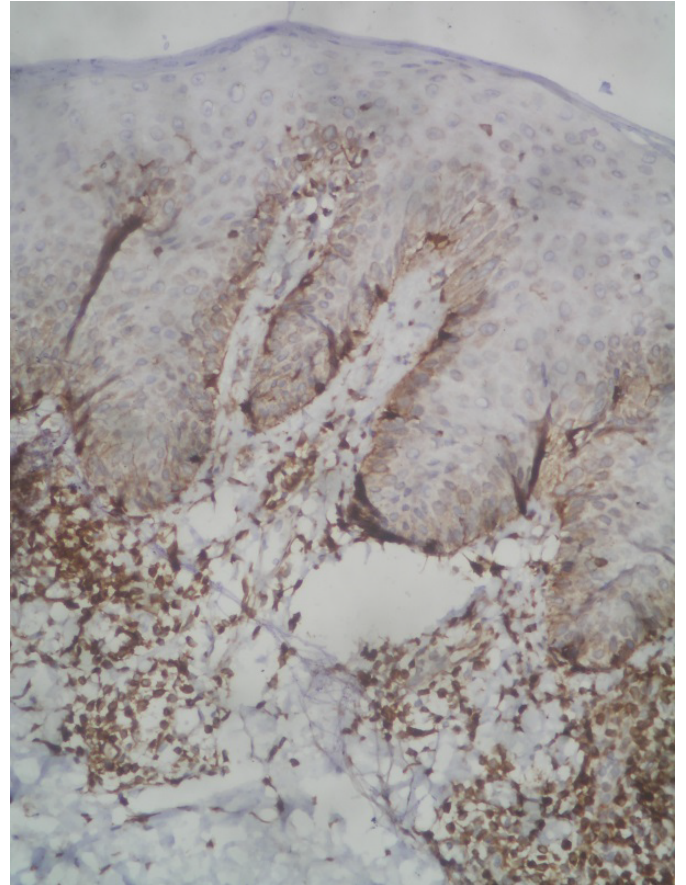


Figure 5: Skin biopsy showing mild to moderate positivity for epidermal and basal cells and intense dermal lymphocytic positivity for bcl2 immunostain at 0 weeks of methotrexate therapy (200X).

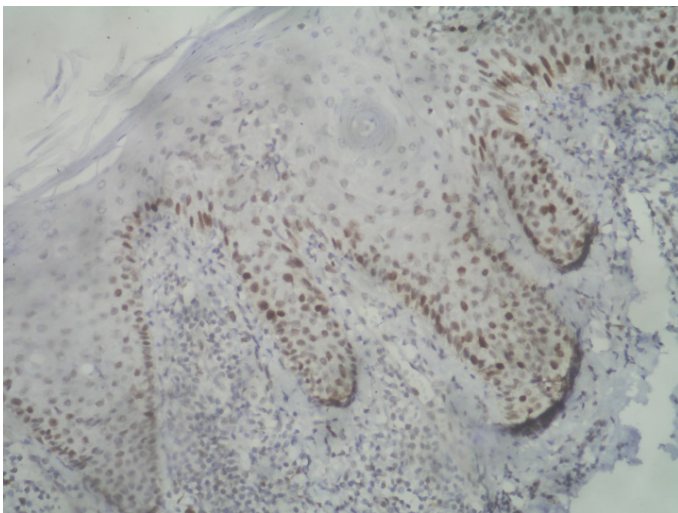


Figure 4: Skin biopsy showing reduced/mild positivity for epidermal, basal cells and dermal lymphocytic for p53 immunostain at 6 weeks of methotrexate therapy (200X).

Saurat et al after 6 and 16 weeks of treatment with methotrexate respectively. ^[14,15]

The characteristic pattern of fully developed psoriasis includes elongation of rete pegs leading to regular acanthosis, edema of the papillary dermis associated with tortuous dilated vessels, thinning of suprapapillary area, decreased thickness of granular layer and exocytosis of neutrophils in the spinous layer (kogoj's pustule) or in the cornified parakeratotic layer

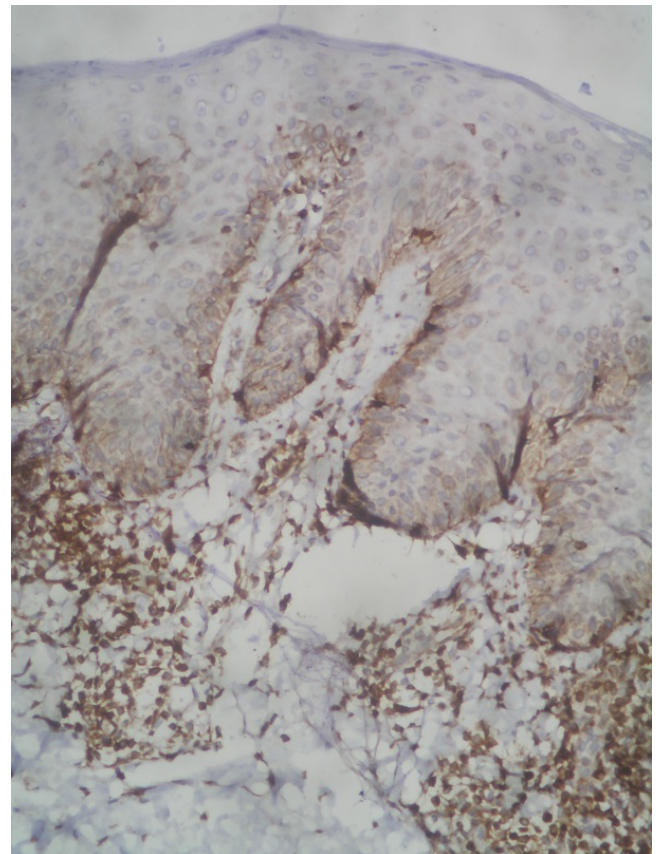


Figure 6: Skin biopsy showing moderate positivity for epidermal, basal cells and dermal lymphocytes for bcl2 immunostain at 2 weeks of methotrexate therapy (200X).

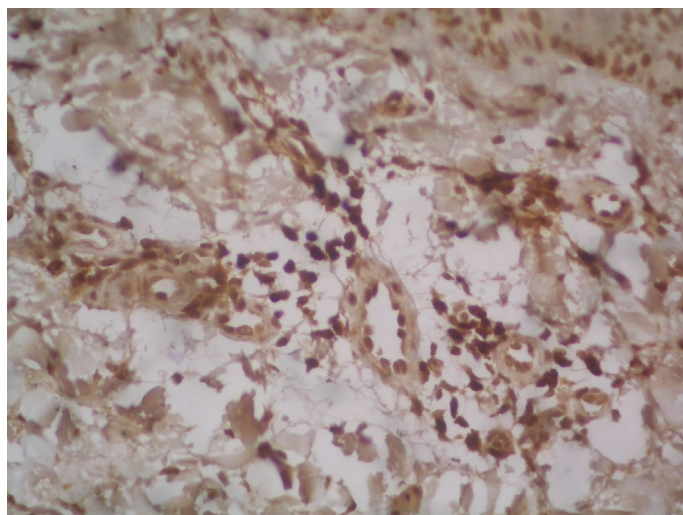


Figure 7: Skin biopsy showing moderate positivity for epidermal and basal cells and intense dermal lymphocytic positivity for bcl2 immunostain at 6 weeks of methotrexate therapy (200X).

(munro microabscesses).^[16] Yazici et al. found a decrease in inflammatory cell infiltrate and progressive loss of elongated rete pegs with the return of a normal stratum corneum in patients after 6 weeks of methotrexate therapy.^[14] In our study, we found that there was an increase in grade 0 cases and a significant reduction in grade 3 cases of psoriasis in all the histomorphological parameters over 0 week to subsequent 2 and 6 weeks.

El-Batawi et al. in a study on expression of Bcl2 and p53 in psoriasis found higher p53 expression in lesional than in non lesional psoriatic skin using monoclonal antibody Do7. They concluded that elevated levels of p53 in psoriatic plaques reflect an important role exerted by this tumor suppressor gene on the development of psoriatic plaques.^[17] Hussein et al. showed that the Bcl2 score in epidermal keratinocytes in normal skin is slightly higher (0.70 ± 0.26) as compared to the psoriatic skin (0.46 ± 0.22) and in inflammatory cells in normal skin, it is lower (2.00 ± 0.00) as compared to the psoriatic skin (3.00 ± 0.39).^[18] El-Domyati et al. found that in psoriatic plaques, Bcl2 was expressed in the keratinocytes of 23.3% cases (mean, 0.3 ± 0.6) and in the lymphocytes of 56.67% cases (mean, 0.8 ± 0.8). In non lesional skin, Bcl2 was expressed in keratinocytes in 46.67% cases (mean, 0.6 ± 0.6) but was not detected in the lymphocytes. The expression was not significantly different in keratinocytes but significantly higher in lymphocytes.^[19] Our results are concordant with the results of few studies and show that Bcl2 was moderately expressed in the control group in epidermal cells (mean, 2.6 ± 0.70), basal cells (Mean, 3.7 ± 0.68) and lymphocytes (mean, 3.67 ± 0.70). At 0 weeks in psoriatic patients, Bcl2 was moderately expressed in epidermal cells (mean, 3.03 ± 0.86) and strongly expressed in basal cells (mean, 4.00 ± 0.80) and lymphocytes (Mean, 4.43 ± 0.85). The expression of Bcl2 in the lymphocytes is significantly higher ($p < 0.05$) in psoriatic skin as compared to the normal skin (control group) but the difference in Bcl2 expression in epidermal and basal cells is statistically insignificant. In the present study, mean expression of Bcl2 in epidermal cells, basal cells and lymphocytes showed significant reduction neither after 2 weeks

($p > 0.05$) nor after 6 weeks ($p > 0.05$) of methotrexate therapy in all the three parameters, keeping 0 week as the baseline.

Some studies have been conducted to look for histological and immunohistochemical changes after giving treatment either in the form of phototherapy or drugs like acitretin, PUVA,^[19] calcipotriol,^[20] infliximab,^[21] anthralin or aloe vera^[21] but very few have been done using methotrexate [Table 4].^[5]

In the present study, histomorphology and expression of p53 and Bcl2 before and after methotrexate therapy in psoriatic patients was studied. All the histomorphological parameters were reduced significantly ($p < 0.05$) after methotrexate therapy. There was also a significant reduction ($p < 0.05$) in p53 expression in epidermal cells, basal cells and lymphocytes. But the expression of Bcl2 was variable in all the three parameters. We can thus safely say that low dose of methotrexate (0.3-0.5 mg/kg) contributes to reduction in epidermal thickness and thereby remains an effective therapy for psoriasis.

Conflict of Interest

All authors disclose that there was no conflict of interest.

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