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Evaluation of psoriasis severity and inflammatory responses under concomitant treatment with methotrexate plus micronutrients for psoriasis vulgaris: a randomized double blind trial

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Abstract

Introduction: We evaluated the effectiveness of concomitant treatment with methotrexate (MTX) plus micronutrients in comparison with monotherapy with MTX only in psoriasis patients. Plasma levels of interleukin-1 beta (IL-1β) and tumor necrosis factor alpha (TNF-α) were also measured and their association with clinical severity was evaluated.
Methods: Thirty psoriasis patients 20 to 50 years old with a PASI score › 10 were divided randomly into two groups. Both groups were given oral methotrexate (0.2–0.3 mg/kg/week) for 12 weeks. In addition, Group B received one tablet of micronutrient supplement daily. Disease severity was calculated using the psoriasis area and severity index (PASI) score before and after 12 weeks. Levels of IL-1β and TNF-α were measured using enzyme-linked immunosorbent assay (ELISA).
Results: We found that 13 (86.6%) patients in Group B and 8 (53.3%) patients in Group A attained a mild PASI score (≤ 10% body involvement). IL-1β and TNF-α levels were significantly decreased in favor of Group B ($p < 0.05$). There was a significant correlation between changes in both IL-1β and TNF-α levels and PASI score after the study ($p < 0.05$).
Conclusion: The results obtained were positive, and therefore double-blind randomized trials with a larger sample size are highly suggested to confirm or reject these results.

Keywords: Psoriasis, methotrexate, tumor necrosis factor alpha, interleukin-1 beta, psoriasis concomitant treatment

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Background

Psoriasis vulgaris is a chronic immune-mediated skin disorder with a global prevalence of 2% (1). The pathogenesis of psoriasis is unknown, although the main reason had been considered a disturbance in biochemical markers. Recently, however, immunological genetics-related disturbances have been widely accepted (2). In psoriasis vulgaris, the affected epidermis is significantly infiltrated with immune cells and produces abnormal cytokines. T cells in psoriatic lesional skin are considered to produce many pro-inflammatory cytokines belonging to type 1 helper cells (Th1) such as interleukin-1beta (IL-1β) and tumor necrosis factor-α (TNF-α) to initiate and/or maintain the cell-mediated keratinocyte hyperplasia in inflammatory lesions (3). These two cytokines as a mediator of the acute phase of inflammation induce the expression of adhesion molecules (ICAM-1) on endothelial cells, which facilitate leucocyte entry into inflammatory sites and promote progression and hyper-proliferation in psoriasis lesions (4, 5).

Methotrexate (MTX) as the gold standard therapy for moderate to severe psoriasis exerts its effects as both an immune-modulatory and antimetabolite agent (6). According to European League against Rheumatism (EULAR) recommendations, MTX therapy as a first-line conventional synthetic disease-modifying antirheumatic drug (csDMARD) therapy is also suggested for moderate to severe psoriasis arthritis (7, 8). Methotrexate applies its immune-modulatory effects by decreasing T cell–mediated inflammation at multiple steps, which causes inhibition of keratinocyte growth and down-regulates the endothelial expression of ICAM-1 and E-selectin (9, 10). MTX-based therapy among psoriasis patients is mentioned because of its excellent efficacy and its availability to patients in low-income countries (11, 12). Beyond the role of MTX therapy for psoriasis, the anti-inflammatory role of micronutrient supplements (MM) in modulating the immune system in psoriasis patients was established previously in the literature (13, 14). Despite milder flare-ups and reduction of scales and erythema under micronutrient consumption (15, 16) the clinical efficacy of MM in psoriasis treatment has been neglected for decades. However, today the increasing numbers of treatment modalities with respect to diet regimen, complementary medicines, and combined therapies for psoriasis patients are controversial among dermatologists and demand the results of approved clinical trials. Moreover, there is still a lack of knowledge about variations of pro-inflammatory cytokines under MTX or micronutrients and their relation to clinical response in psoriasis patients. Therefore, this study investigates the clinical efficacy of a combined therapy for the first time: micronutrients plus MTX versus MTX only and its relation to pro-inflammatory mediators including IL-1β and TNF-α in psoriasis vulgaris patients whose disease was confirmed by punch biopsy prior to admission.

Patients and methods

Study participants

In this double blind randomized trial, Asian patients with psoriasis vulgaris that visited our Dermatology Department at Mashhad University Medical University in Mashhad, Iran were recruited. This trial was approved by the Ethics Committee of Mashhad University of Medical Sciences in Mashhad, Iran. Written informed consent was obtained from all patients. The diagnosis of psoriatic vulgaris was performed by punch biopsy after receiving separate written informed consent. This trial was registered under the

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Iranian Registry of Clinical Trials (IRCT; no. 2014012016275N1). The inclusion criteria were patients 20 to 50 years old and suffering from plaque type psoriasis with a PASI score higher than 10 that had not received methotrexate, phototherapy, and systemic MTX therapy for at least 2 months.

Exclusion criteria were patients under 20 years old, other forms of psoriasis except psoriasis vulgaris, those that wanted to leave the study for any reason, and patients with acne vulgaris, kidney disease, type one diabetes mellitus, cardiovascular disease, pregnancy, familial hyperlipidemia, and a history of liver disease and/or variations in hepatic enzymes during the study (more than 2.5 times the normal level). In addition, patients that self-administered micronutrients and over-the-counter medicines, patients on a special diet, and those that consumed alcohol or alcoholic beverages during the study and the prior 2 months were also excluded. For preventing drug-drug interactions with MTX, patients that had used any other medications, including antibiotics, antidepressants, and non-steroidal anti-inflammatory drugs, were excluded. In addition, based on previous research on yellow fever vaccines' contraindication for immune-compromised patients, we removed patients with yellow fever vaccines (17).

Sample size

We considered psoriasis area and severity index (PASI)-75 for efficacy of treatment with a defined reduction in PASI score by 75% from baseline (18). Then, regarding the 75% cure rate of MTX in psoriasis patients that achieved PASI-75 at week 12 (18), 95% power, and a 5% two-sided type I error, 11 subjects were required in each group. After this, due to a 20% loss to follow-up, this number was increased to 15 patients for each group (Fig. 1).

Study design and blinding

Initially, patients were divided into two different modalities of treatment by a dermatologist based on odd and even days of referral time. The patients that referred on odd days were assigned to Group A and those that referred on even days were assigned to Group B. Patients in both groups were treated with 7.5 to 15 mg of MTX per week for 12 weeks (dose of 0.2–0.3 mg/kg/week) according to the standard protocol of MTX consumption (18). During systemic treatment, no concomitant antipsoriatic therapy was permitted, with the exception of emollients. Furthermore, Group B received one

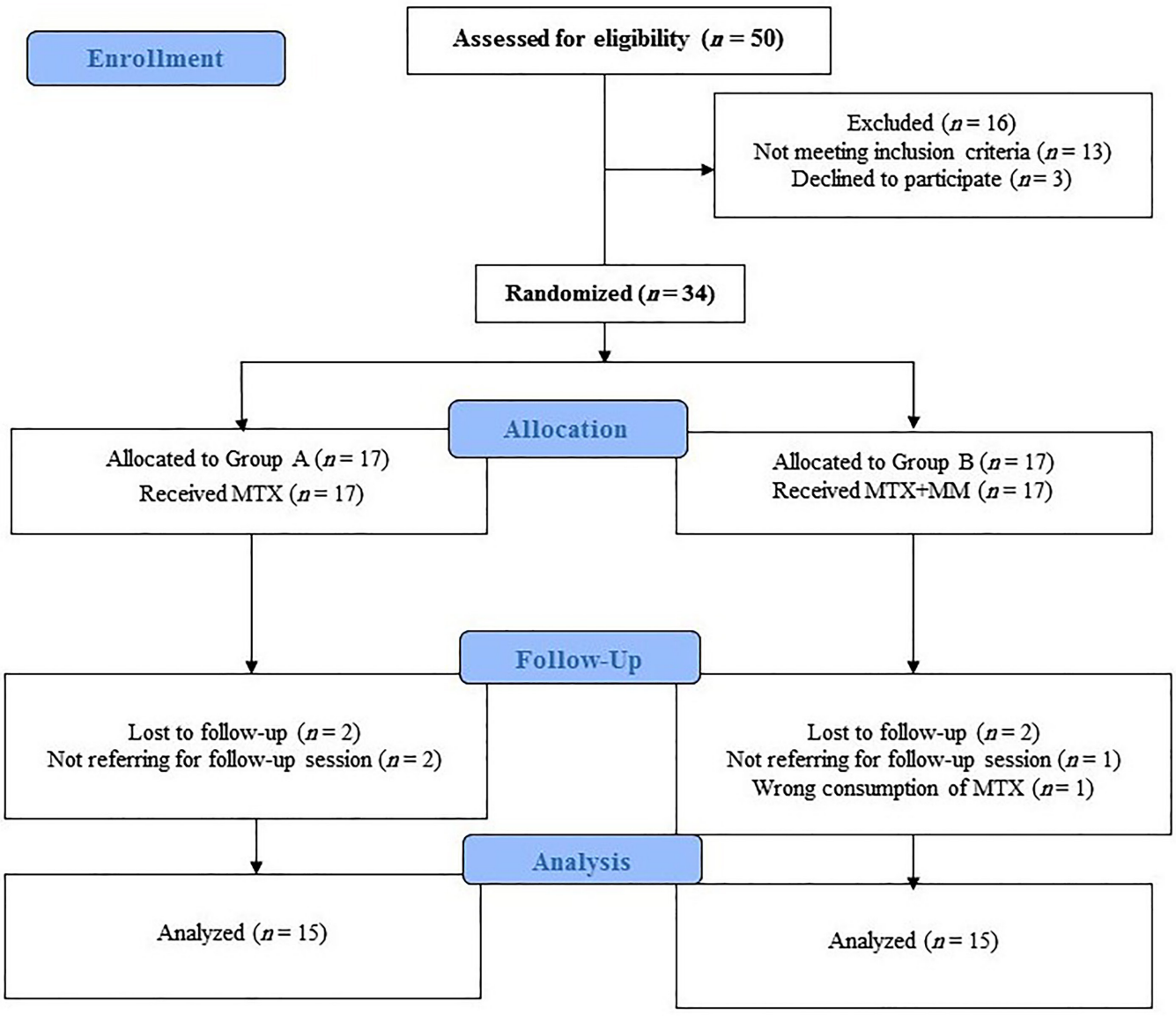

Figure 1 | Flow diagram of patients studied in a randomized trial according to the CONSORT 2010 Flow Diagram.

tablet of MM (Immunace, Vitabiotics Ltd., London, UK). The dosages used in this MM for daily consumption (Supp1) were higher than the recommended daily allowances (RDA) for healthy individuals (19). It was logical that psoriasis patients have greater requirements for micronutrients (16). In addition, folic acid was given (5 mg once daily) to all patients except on the day of MTX consumption.

Furthermore, patients were blinded for treatment groups due to receiving their therapies with masked trademarks and, as mentioned, due to different days of their referral.

Safety of treatment

Patients were requested to report any occurrence of undesired adverse effects to the dermatologist, including burning, itching, redness, and chapping during the treatment period. Moreover, the serum level of liver function tests (LFT), including measuring alanine aminotransferase (ALT or SGPT), aspartate aminotransferase (AST or SGOT), and alkaline phosphatase (ALP) enzymes, was measured and recorded at baseline and during follow-up sessions. In addition, we evaluated erythrocyte sedimentation rate (ESR) and high-sensitive C-reactive protein (hS-CRP) because its probable elevated levels could be connected with the medication used (20) before and during the study.

Efficacy of treatment

Severity of disease was measured objectively using the PASI scoring system (21) before and at the end of 12 weeks of therapy by three independent investigators that were not aware of the treatment groups. Regarding the PASI score, the psoriasis was categorized as mild (PASI ≤ 10), moderate (10 > PASI ≤ 30), or severe (30 > PASI ≤ 72). Furthermore, PASI-75 was measured as the effectiveness indicator of this study process.

Inflammatory cytokine assay

We collected 5 cc blood samples from the participants before and after the end of the trial. Freshly isolated heparinized peripheral blood samples were centrifuged immediately for 10 min at 500 g, and then the plasma was separated. Then the plasma concentrations of IL-1β and TNF-α level were measured using the enzyme-linked immunosorbent assay (ELISA) technique with this kit (eBioscience, San Diego, CA, USA) according to the manufacturer's instructions. These ELISA kits contain reagents including monoclonal antibodies to human IL-1β or human TNF-α , biotin-conjugate anti-human monoclonal antibody, streptavidin-HRP, sample diluent, amplification reagent (ethyl alcohol), wash buffers, substrate solution (tetramethyl-benzidine), and stop solution (phosphoric acid). The absorbance was read at 450 nm at room temperature.

Data collection and statistical analysis

We recorded the history of the patients, including personal history (name, age, sex, and contact number) and history of the disease, including age of onset, course, previous treatment, and any other systemic infection. The data were analyzed statistically using SPSS software version 16. The descriptive data were summarized as mean plus, minus standard deviation (SD), standard error of mean (SE), and percent of patients. First, the normality of data was checked using a one-sample Kolmogorov–Simonov test. An independent t-test and Mann–Whitney test for parametric and non-parametric data, respectively, were used to compare the cytokine status and PASI score classification between the psoriasis groups. Analysis of variance using an ANOVA test was used to compare clinical scoring between the groups. A Pearson correlation test was performed to evaluate the correlation between changes in PASI score and levels of IL-1β and TNF-α before and after the study. Because of considering PASI-75 to represent efficacy of the treatment, this comparison between the two groups was performed using the last observation carried forward (LOCF) approach, which included all 34 patients enrolled at the beginning of the trial and considered patients that were lost to follow-up periods.

Results

Patient details

In line with the inclusion criteria, 34 psoriasis patients were entered in the analysis (Fig. 1). However, four patients could not complete the study due to not referring for follow-up sessions and one patient forgetting to take MTX for 2 weeks. The demographic and PAS-75 clinical outcome characteristics of the patients are presented in Table 1. There were 18 men and 12 women, and the ages of the patients ranged from 21 to 70 years (mean ± SD, 46.4 ± 14.1 years). There was no difference between Group A and Group B with respect to age, age at onset of disease, and weight ($p > 0.05$, Table 1). Based on the similar weight of patients between the two groups, the dosage of MTX was similar between the two groups ($p > 0.05$, Table 1). There were no reports of unwanted side effects, discomfort, or significant attenuation of liver function test enzymes in both groups treated (more than 2.5 times the normal level; Table 2). In addition, there were no significant differences regarding changes in hs-CRP and ESR during the study.

Table 1 | Demographic information and comparison of the therapeutic response rates in the groups studied using the intention-to-treat approach and per-protocol analysis of the groups.

Variables		Group A	Group B	P-value
Male/Female (N)		9 (30%) / 6 (20%)	9 (30%) / 6 (20%)	–
Age (years)		38.60 ± 11.59	38.46 ± 10.89	*0.97
Age onset of disease (years)		31.73 ± 11.71	27.93 ± 12.59	*0.40
Weight (kg)		67.5 ± 5.37	61.90 ± 12.87	0.13
MTX dosage (mg/week)		13.5 ± 1.07	12.38 ± 2.57	0.13
Analytical assumption[a]	Per-protocol	11/15 (73.3%)	6/15 (40.0%)	§0.05
	LOCF	11/17 (64.7%)	6/17 (35.3%)	§0.08

The Group A and Group B definitions are mentioned within the text. The *p-value is calculated using an independent sample test and chi square test, and a p-value less than 0.05 is considered significant. [a]Per protocol = analysis excluding patients that were lost to follow-up, LOCF = analysis including patients lost to follow-up throughout the study that were considered to have experienced treatment failure

Clinical scoring

The severity of disease corresponding to the PASI score was evaluated for all participants during the study period. The PASI score decreased significantly in both treatment groups from the baseline to the end of 12 weeks of treatment ($p = 0.001$, Table 3). Although both groups had a similar PASI score ($p = 0.69$) before the study, the PASI score decreased significantly differently between the groups after 12 weeks ($p = 0.04$). The analysis showed that this significant reduction was to the benefit of Group B compared to Group A ($p = 0.045$). Moreover, the PASI score classification shows that all of the patients with moderate to severe psoriasis in group B before the study attained mild psoriasis after the study, except for two patients (13.3%), who attained a moderate score after the study (Table 3, $p = 0.04$). In contrast, eight (53.3%), six (40.0%), and one (6.7%) of the patients in after 12 weeks of treatment.

Considering the overall efficacy of treatment at week 12 based on per-protocol analysis, we found that 11 (73.3%) and six (40.0%) patients in Group B and Group A attained PASI-75 (p = 0.05), respectively. Moreover, after LOCF analysis, the same results were seen with a marginal significance between the two study groups (p = 0.08, Table 1).

Plasma levels of IL-1β and PASI score

Figure 2a compares the IL-1β plasma concentration before and after the study between Groups A and B. This figure shows that baseline level of IL-1β was similar among the patients (p = 0.09). However, IL-1β decreased significantly in both groups during the study (Fig. 2a). Nevertheless, analysis showed that after 12 weeks the IL-1β levels of Group B were significantly lower than the IL-1β levels of Group A (p = 0.04).

The analysis of the correlation of variations in IL-1β and PASI score (Fig. 3) showed that there was no significant correlation between them at week 0 (Fig. 3a, p > 0.05) whereas this correlation was significant at week 12 (Fig. 3b, r = 0.42, p = 0.02). Table 4 shows the relation between PASI score classification (mild, moderate, and severe) and IL-1β levels before and after the study. During the study, patients with severe and moderate psoriasis experienced higher levels of IL-1β than patients with a mild PASI score, although this was not significant (p > 0.05). However, after the study, patients with mild and moderate psoriasis in Group B had lower IL-1β levels than patients with similar severities in Group A.

Table 2 | **Evaluation of biochemical tests between the two groups during the study.**

Group	SGPT, week 0	SGPT, week 12	SGOT, week 0	SGOT, week 12	ALP, week 0	ALP, week12	s-CRP week 0 pg/ml	s-CRP week 12 pg/ml	ESR week 0	ESR week 12
A	24	40	32	45	190	185	2.0	1.5	8.0	8.0
A	15	16	18	20	196	208	1.5	1.45	8.0	9.0
A	46	50	36	46	208	211	1.8	1.78	5.0	6.0
A	19	20	22	15	306	293	2.6	2.0	10.0	10.0
A	19	49	59	65	130	180	2.42	2.15	4.0	3.0
A	19	33	18	280	267	285	2.8	1.5	13.0	13.0
A	22	35	18	28	195	230	2.9	2.0	14.0	14.0
A	40	49	43	40	250	300	3.0	1.98	12.0	12.0
A	31	36	24	24	219	224	2.3	1.85	19.0	18.0
A	9	12	11	12	191	200	2.75	1.59	8.0	8.0
A	29	32	30	35	300	320	2.32	1.89	12.0	12.0
A	20	22	15	19	190	200	2.48	2.0	9.0	10.0
A	40	45	74	50	172	180	2.98	1.7	6.0	6.0
A	61	87	37	50	178	181	2.87	2.5	10.0	11.0
A	18	30	20	28	170	200	2.99	2.4	12.0	12.0
B	27	27	22	22	140	140	3.12	2.0	4.0	4.0
B	26	30	21	35	208	211	4.0	2.1	10.0	9.0
B	35	69	24	34	185	220	2.21	1.6	8.0	9.0
B	40	50	43	45	246	300	2.23	1.5	15.0	15.0
B	15	49	13	34	155	180	2.01	1.8	12.0	12.0
B	18	34	21	30	137	180	2.2	1.5	9.0	8.0
B	9	13	11	12	290	302	2.0	1.54	4.0	5.0
B	22	35	16	28	206	178	2.15	1.85	9.0	9.0
B	60	70	25	40	135	145	2.19	1.85	10.0	10.0
B	24	34	16	26	281	300	1.98	1.4	2.0	3.0
B	10	17	12	16	139	191	2.46	1.78	10.0	10.0
B	15	18	19	17	162	105	2.38	1.9	2.0	2.0
B	14	16	25	23	159	165	2.94	1.86	2.0	2.0
B	30	33.5	26	33	250	279	2.54	1.94	12.0	12.0
B	15	21	16	18	160	165	2.35	1.98	5.0	5.0
*P-value	0.49	0.69	0.06	0.19	0.28	0.28	0.73	0.26	0.11	0.09

The Group A and Group B definitions are mentioned within the text. SGPT, SGOT, and ALP refer to alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase. The *p-value under the independent sample test compares the related variables between Group A and Group B. A p-value less than 0.05 was considered significant.

Table 3 | **Comparison of clinical psoriasis scoring in the groups studied.**

Variable		Before study (mean ± SD)	After study (mean ± SD)	P-value*
Group A, PASI		30.23 ± 10.87	10.86 ± 9.84	0.001
Group B, PASI		31.80 ± 10.57	5.50 ± 3.82	0.001
P-value§		0.69	0.04	§0.04
Group A N (%)	Mild	–	8 (53.3)	0.11
	Moderate	10 (66.6)	6 (40.0)	
	Severe	5 (33.3)	1 (6.7)	
Group B N (%)	Mild	–	13 (86.6)	0.04
	Moderate	7 (46.6)	2 (13.3)	
	Severe	8 (53.3)	–	

SD = standard deviation, N = number, p-value* and p-value§ compare differences in each group (inter-evaluation) and between groups (intra-evaluation) before and after the study and calculated using the ANOVA test. A p-value less than 0.05 is considered significant.

Plasma levels of TNF-α and PASI score

TNF-α plasma concentration before and after study between Groups A and B is shown in Fig. 2b. Both groups had similar TNF-α at the baseline (p = 0.80). TNF-α levels decreased significantly in both groups during the study (p < 0.05, Fig. 2b). The analysis showed that after the study levels of TNF-α in Group B were significantly lower than in Group A (p = 0.03).

There was no significant correlation between plasma concentrations of TNF-α at baseline of the study (Fig. 4a, p > 0.05), although this correlation was significant at the end of week 12 (Fig. 4b, r = 0.56, p = 0.02). The relation between the PASI score classification and TNF-α level is presented in Table 4. Patients with severities of mild, moderate, and severe psoriasis experienced the lowest non-significant levels of TNF-α during the study (p > 0.05).

Table 4 | **Relation of clinical psoriasis scoring (mild, moderate, and severe) and cytokines (IL-1β and TNF-α) for the two groups studied before and after the study.**

Cytokine	Group	Severity	Group A (mean ± SE)	Group B (mean ± SE)	P-value
IL-1β (pg/ml)	Before	Moderate	10.62 ± 1.37	10.70 ± 0.32	0.96
		Severe	11.48 ± 1.58	11.05 ± 0.53	0.76
	After	Mild	9.62 ± 1.16	8.23 ± 0.30	0.22
		Moderate	12.27 ± 1.88	8.62 ± 0.30	0.12
		Severe	13.27 ± 0.8	–	–
TNFα (pg/ml)	Before	Moderate	4.01 ± 0.57	4.34 ± 1.57	0.34
		Severe	4.34 ± 1.57	3.60 ± 0.82	0.68
	After	Mild	2.03 ± 0.62	0.98 ± 0.27	0.11
		Moderate	3.61 ± 1.27	1.24 ± 0.02	0.73
		Severe	4.60 ± 0.07	–	–

SE = standard error of mean. A p-value less than 0.05 is considered significant

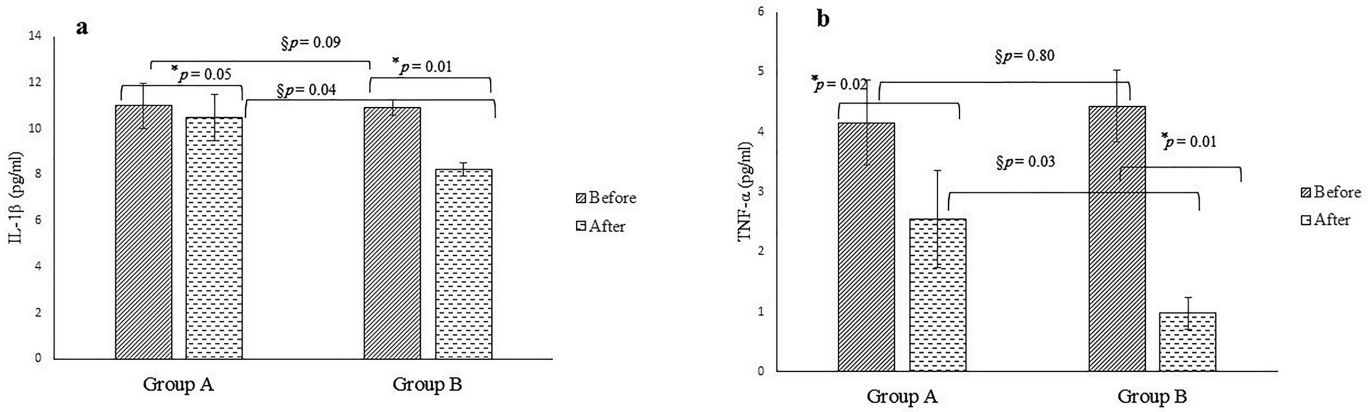

Figure 2 | Comparison of IL-1β (a) and TNF-α (b) levels during study. The numbers in each bar compare the differences between inter groups (*p-value) and intra groups (§p-value) before and after the study. A p-value less than 0.05 is considered significant.

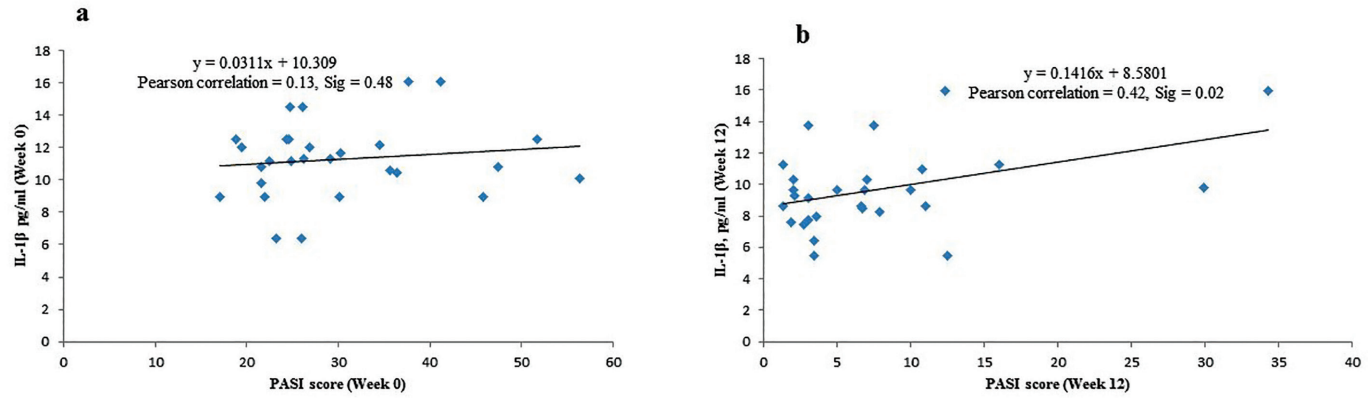

Figure 3 | Correlation between levels of IL-1β before (a) and after (b) the study. A p-value less than 0.05 is considered significant.

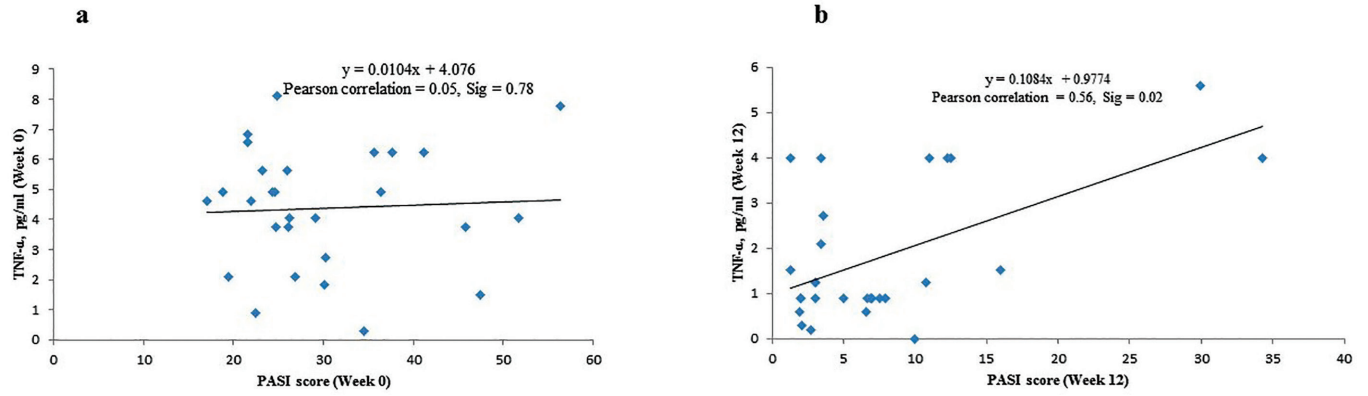

Figure 4 | Correlation between levels of TNF-α before (a) and after (b) the study. A p-value less than 0.05 is considered significant.

Discussion

Psoriasis is a common papulo-squamous disorder that responds to therapy with various results reported; it continues to challenge dermatologists because of various therapies. In this study we devised a novel risk-free combinational treatment modality using MTX and MM, which caused a notable decrease in the psoriasis severity score without any adverse effect, along with significant attenuation of pro-inflammatory cytokines (IL-1β and TNF-α) compared to patients treated with MTX alone.

The efficacy of MTX treatment was reported to be 24% to 100% among the psoriasis patients that achieved PASI-75 during the 12 weeks with a dosage of 7.5 to 15 mg weekly (18). However, under similar situations, we found that 73% of Group B and 40% of Group A patients reached PASI-75 at week 12. There are many coupled treatment modalities for psoriasis (22); however, a few trials combined MTX with micronutrients for psoriasis therapy (23, 24). In this study, 13 (86.6%) of patients with MTX plus MM therapy attained a mild PASI score (less than 10% BSA or 90% improvement). In contrast, eight (53.3%) patients with MTX only attained a mild PASI score. Similar to our findings, in 1997 Katz reported that combined use of MTX and vitamin D analogue caused an estimated 75% or greater lesion improvement in 75% to 100% of patients (23). Previous reports found that micronutrients such as vitamin D and vitamin A analogues, vitamin E, vitamin B12, selenium, and zinc provide an alternative treatment in psoriasis vulgaris patients (15, 16, 25). Therefore, considering the same baseline PASI score among the patients studied, we attributed this higher reduction of PASI score of Group B in comparison to Group A to the effect of MM, which was coupled with the efficacy of MTX in psoriasis treatment. However, there is not enough relevant literature on linking the use of MM and related mechanisms in improving psoriasis lesions. The mechanisms of MM in improving psoriasis lesions might be attributed to their roles in immune response. Is probable that MM improve T-lymphocyte function or one or more components of the innate immune system and block the activity of inflammatory cytokines in psoriasis patients. Similarly, the role of vitamin A in regulating several elements of the immune response (26), or suppressing the pro-inflammatory cytokines of psoriasis such as IL- 1β, IL-17, and TNF-α, has been reported before (27). Moreover, the established clinical efficacy of vitamin D3 was characterized as suppressing the IL-1 family, IL-12/23 p40, and TNF-α in psoriatic lesions (27–29). Moreover, the roles of selenium, vitamin E, and vitamin B12 supplementation in improving indices of cell-mediated immunity, diminishing the IL-1 family and TNF-α, and control of the immune pathogenesis response in psoriasis lesions were documented previously (30–32). In addition, the role of dietary zinc in activating NF-κB, expression of IL-1β and TNF-α, and neutrophil infiltration during the early stages of cutaneous wound healing was also confirmed previously (33).

Cytokines play an important role in the pathological pathway of keratinocyte proliferation and have pleiotropic effects on epithelial cells in vitro and in vivo (22). The role of IL-1β as an important factor of inflammation in various skin diseases including psoriasis was confirmed (27). We found that the IL-1β levels decreased significantly in both groups during the study. Similar to our findings, a study of psoriasis patients by Tamilselvi et al. (34) showed that IL-1β levels decreased significantly after MTX treatment. However, IL-1β was higher in severe psoriasis patients than in patients with moderate and mild severities. Similar to our findings, a significant negative association was found between IL-1β level and disease severity in patients treated with MTX alone (35). A previous investigation explained that the decrease in IL-1β levels with MTX is due to the inhibitory effect of MTX on 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) transformylase (ATIC). ATIC then accumulates and increases adenosine release into circulation. Furthermore, extracellular adenosine does increase cAMP, which inhibits the production of pro-inflammatory cytokine IL-1β (35). On the other hand, MTX reduces IL-1β levels by decreasing the infiltration of lymphocytes and monocytes or blocking its binding to the IL-1 receptor (36). At the end of trial, we found a 2.67 pg/ml and 0.52 pg/ml significant decrease in IL-1β levels in Group B and Group A, respectively. This could be explained based on the aforementioned reports on the role of MM in modulating psoriasis cutaneous inflammatory reactions, at least in part, through suppression of gene expression of IL-1 family members (26–33). Therefore, in support of previous work, this higher attenuation of IL-1β in favor of Group B could be attributed to coupling the anti-inflammatory role of MTX and MM in the group.

Furthermore, we found a significant decrease in TNF-α level during this study. Similar research on the effect of MTX on TNF-α level found that the elevated serum TNF-α in psoriasis patients decreased significantly after MTX treatment (37). They concluded that decreasing inflammatory caspase and pro-inflammatory cytokines with MTX inhibits the Th1 response in psoriasis. This point emphasizes the therapeutic effect of MTX in controlling the immune-pathogenesis of psoriasis. However, like IL-1β, higher attenuation of TNF-α in Group B than in Group A (3.45 pg/ml compared to 1.60 pg/ml) corresponds to a greater decrease in inflammation in this group. This might be due to coupling the immune-modulatory roles of MM (13) and MTX to suppress TNF-α levels and consequently to inhibit inflammation. Tumor necrosis factor (TNF) is a key mediator of cutaneous inflammation and is characterized as an important protagonist of skin immunity (38). TNF-α antagonists have been successfully utilized to treat pustular psoriasis. This successful treatment is not only related to a decrease in TNF-α level. It has been explained that TNF-α not only regulates the antigen-presenting ability of DCs but also promotes infiltration of T cells (39).

In conclusion, decreasing pro-inflammatory cytokines (IL-1β and TNF-α) with MTX may inhibit the Th1 response in psoriasis. This shows the therapeutic effect of MTX in controlling the immune-pathogenesis of psoriasis. Moreover, we found a significant decrease in IL-1β and TNF-α through better clinical response in patients that were treated with MTX plus MM compared to those treated with MTX only. Therefore, the authors recommend the combined use of MTX plus MM for better psoriasis control. To this end, this study has opened an avenue for more investigations in the future.

Study limitations

There are some limitations for this study, including the small sample size, which clearly limits the evidence it offers. We did not perform titration of MTX in an early phase of treatment and during the study due to the need for more laboratory instruments (HPLC using fluorometric detection) and the major expense of this test for the patients in our study area. Therefore, we suggest that further studies measure MTX titration for verification and for a more accurate and sensitive description of MTX efficacy in psoriasis vulgaris patients. In addition, a chloride blood measurement test (CL) for subsequent probable kidney failure in patients with MTX therapy could be suggested. Furthermore, we recommend that future studies contain a larger number of psoriasis patients to confirm the clinical effects of MTX plus MM.

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