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ARTICLE



Effect of Cholecalciferol Supplementation on Treatment Response and IL-10 Level in Vitamin D Deficient *Parthenium* Dermatitis Patients: A Randomized Double-Blind Placebo-Controlled Trial

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ABSTRACT

Allergic contact dermatitis following exposure to *Parthenium* is a chronic disease associated with frequent relapses and significant disturbance in the quality of life. The affected patients have lower circulating levels and decreased expression of IL-10. Hence, measures to increase its level may enhance the therapeutic outcome. The clinical trial was undertaken to evaluate the effect of cholecalciferol supplementation on treatment response and IL-10 level in *Parthenium* dermatitis patients with vitamin D deficiency. A total of 72 patients were recruited and randomized to receive either cholecalciferol tablet 60,000 IU per week or matching placebo for 8 weeks with standard background treatment. Eczema Area Severity Index (EASI) and Dermatology Life Quality Index (DLQI) were assessed at baseline, 4 weeks, and 8 weeks while IL-10 and serum 25-hydroxyvitamin D levels were measured at baseline and 8 weeks. Levels of 25-hydroxyvitamin D and IL-10 showed a significant rise in both placebo and vitamin D groups following the intervention. The relatively higher increase in IL-10 level observed in the vitamin D group was statistically insignificant compared to placebo group. Significant reduction in EASI, as well as DLQI scores, was noted after 1 and 2 months, but the reduction in these scales was not significantly different between the groups. Cholecalciferol supplementation for 2 months did not reduce the disease severity in clinically diagnosed *Parthenium* dermatitis patients. However, treatment initiation significantly improved plasma IL-10 levels after 2 months in both placebo and cholecalciferol groups.

KEYWORDS

allergic contact dermatitis; cholecalciferol; *Parthenium*; IL-10 cytokine; vitamin D

Introduction

The growth and spread of weed plant species are of serious concern because of their ability to cause environmental and public health hazards. *Parthenium hysterophorus*,

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Trial registry URL: <http://ctri.nic.in/Clinicaltrials/login.php>

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which belongs to Compositae (Asteraceae) family, is one of the most dangerous weeds in the world. The weed has spread across the continents of Asia, Africa, North America, South America, and Australia. In the Indian subcontinent, *Parthenium* has spread over 5 million acres of land area, posing biodiversity and health threats. The plant is known by many names such as congress grass/weed, bitter weed, carrot weed, feverfew, chatak chandani, and gajar ghas; it is also called the “scourge of India” (Bhateria and Renu 2015; Lakshmi and Srinivas 2007).

The active constituents of *Parthenium* are sesquiterpene lactones, volatile oils, phenolic derivative, and flavonoid group of chemicals. Parthenin is the major sesquiterpene lactone. Parthenin along with hymenin, ambrosin, hysterin, tetraeurin A, and coronophilin are the major allergy-producing contents (Sharma and Verma 2012). Exposure to *Parthenium* plant (dried plant, leaf, flower, stem, root, and pollen) can induce allergic hypersensitivity, which causes eczema, allergic rhinitis, asthma, skin blisters, and dark spots. Dermatitis is the common health problem seen among exposed individuals and is commonly mentioned as *Parthenium* dermatitis (Patel 2011; Sharma et al. 2013).

The allergic dermatitis condition is observed in 4.5% of the population exposed to the weed’s allergens at their occupational area and 0.7%–1.4% of the general public in India (Sharma et al. 2005). *Parthenium* is observed to be the causative agent for about 40% of the cases attending the contact dermatitis clinics in India (Sharma 2001). The contact dermatitis occurs following exposure to allergens such as sesquiterpene lactones and significantly affects the quality of life. The condition is explained to be a combined type IV and type I hypersensitivity reaction, which can manifest as classical airborne, chronic actinic, or mixed pattern (Akhtar et al. 2010a, 2010b; Sharma and Verma 2012).

Significantly elevated levels of proinflammatory cytokines, as well as decreased levels of anti-inflammatory cytokines, have been observed in *Parthenium* dermatitis patients compared to healthy controls, suggesting the role of imbalance between proinflammatory and anti-inflammatory cytokines in its pathogenesis (Akhtar et al. 2010a, 2010b). Interleukin 10 (IL-10), also known as cytokine synthesis inhibitory factor (CSIF), is an important anti-inflammatory cytokine involved in innate immune regulation. It acts through inhibition of proinflammatory cytokines and modulation of T-cell response. *Parthenium* dermatitis patients were found to have not only lower circulating levels of IL-10 but also its decreased expression. Similarly, mutations resulting in lower IL-10 levels have been shown to be associated with increased risk for *Parthenium* dermatitis (Khatri et al. 2011). Increase in IL-10 level has been found to shift the T-cell response from allergic type 2 T helper cells (Th2 cell) to nonallergic type 1 T helper cells (Th1 cell) through the action of regulatory T cells (James and Durham 2008).

Although steroids are the mainstay of treatment for this condition, currently steroid-sparing immunosuppressants such as azathioprine, methotrexate, cyclosporine, and so on are commonly used to avoid steroid-related complications. Although complete remission is attained in 3 months, treatment with these agents needs to be continued for a period of 6 months to prevent relapse (Sharma et al. 2013; Sharma and Verma 2012). However, immunosuppressants being narrow therapeutic drugs, they have increased the risk of causing adverse effects such as opportunistic infections, bone marrow suppression, and organ toxicities (Krensky et al. 2018; Lake and Briggs 2018). Since the condition is associated with lower anti-inflammatory cytokine IL-10 level, measures

to increase its level may enhance the therapeutic outcome and help to reduce the duration of immunosuppressive treatment.

Vitamin D, the sunshine hormone, apart from playing its physiological role in bone mineral homeostasis, has been found to have various pleiotropic actions. Vitamin D deficiency is being considered as a modern-day pandemic, affecting almost one-third of world's population (Hilger et al. 2014). The deficiency is common in India in all age groups of both genders with the frequency of 70%–90% (Harinarayan and Joshi 2009). The deficient status has been associated with various consequences such as osteoporosis, bone fracture, allergy, asthma, infections, cardiovascular risk, and malignancies (Holick et al. 2011; Holvik et al. 2013; Jacobs et al. 2016). In preclinical and clinical studies, 1,25-dihydroxy vitamin D₃ has been shown to induce IL-10 expression and its production by immune cells (Heine et al. 2008). With this background, the present study was designed to generate evidence toward the effect of cholecalciferol supplementation on IL-10 levels and treatment response in *Parthenium* dermatitis patients with vitamin D deficiency.

Materials and methods

Study setting

The study protocol was approved by the Institutional Ethics Committee (JIP/IEC/2015/20/725) and the trial was prospectively registered with Clinical Trials Registry - India (CTRI/2016/02/006626). Patients attending eczema clinic at the outpatient Department of Dermatology, JIPMER, were screened for eligibility during the study period from March 2016 to December 2017. Clinically diagnosed *Parthenium* dermatitis patients of either gender, age between 30 and 60 yrs, with serum vitamin D level between 10 and 20 ng/ml, and to be started on systemic treatment with azathioprine were included in the trial. Pregnant and lactating women, individuals already on vitamin D supplementation, patients who had participated in other trials within the past 3 months, and individuals with liver or renal dysfunction were excluded.

Randomization and masking

The study was designed as a randomized double-blind placebo-controlled trial, where the eligible patients were randomized to receive either cholecalciferol tablet 60,000 IU or matching placebo per week for 2 months in the ratio of 1:1 as an add-on to the standard background therapy. The patients were assigned the intervention using block randomization with varied block sizes of 8 and 6, and allocation concealment was done using sequentially numbered opaque sealed envelope (SNOSE) technique. Randomization and allocation concealment were done by the principal investigator's supervisor who did not come in contact with study participants. The principal investigator was involved in screening and recruitment of patients. Both study participants and principal investigator were blinded for the intervention. Blinding was revealed after completion of study and approval of statistical analysis by the supervisor.

Sample size estimation

The sample size was calculated using PS - Power and Sample Size Calculation, version 3.0.12. A sample size of 34 in each group was calculated for the expected difference of 3.2 in mean EASI score between placebo and cholecalciferol groups (Camargo et al. 2014), within-group standard deviation of 4, with a power of 90% and for the level of significance at 0.05. Considering the expected dropout rate of 20% and nonadherence of 10%, the final sample size was estimated to be 45 in each group.

Study procedure

After initial screening for vitamin D deficiency, eligible patients were randomized to receive one of the interventions in addition to the standard background treatment. The participants in both groups were advised regarding the preventive measures to be followed. At baseline, general examination, disease severity scoring (EASI), and quality-of-life scoring (DLQI) were done and 6 ml of blood was collected for IL-10 assay and genotyping. After 2 months of intervention, IL-10 and serum 25-hydroxyvitamin D [25-(OH)D] levels were again measured. Disease severity scoring, quality of life, and adherence to therapy were assessed at baseline and 1 month and 2 months after completion of the intervention. After the intervention period, telephone follow-up was done to monitor the occurrence of relapse and adverse effects.

Serum 25-hydroxyvitamin D measurement

Serum 25-(OH)D levels were measured by chemiluminescence assay according to manufacturer's (SIEMENS – ADVIA CENTAUR XP Immunoassay system) instructions. The assay was standardized using isotope dilution – liquid chromatography – tandem mass spectrometry (ID-LC/MS/MS) 25-(OH)D as reference. The assay procedure was calibrated with standards during each run. The lower limit of quantification was 4.2 ng/ml, and the detection range was up to 150 ng/ml.

IL-10 measurement by ELISA

The IL-10 level in plasma was measured by sandwich enzyme-linked immuno-sorbent assay (ELISA) method as per manufacturer's (Raybio) instructions. Standard concentrations were prepared as per the instructions. The standard and samples were run in duplicates, and average absorbance values were taken for the calculation.

Disease severity and quality of life

EASI scoring was used to assess the severity of dermatitis among *Parthenium* dermatitis patients. The scoring assesses the severity of signs such as erythema, edema/papulation, excoriation, and lichenification along with body region involved and the extent of involvement (Leshem et al. 2015; Zhao et al. 2017). The disease severity can be graded according to EASI score as clear, almost clear, mild, moderate, severe, and very severe according with scores of 0, 0.1–1, 1.1–7, 7.1–21, 21.1–50, and 50.1–72, respectively.

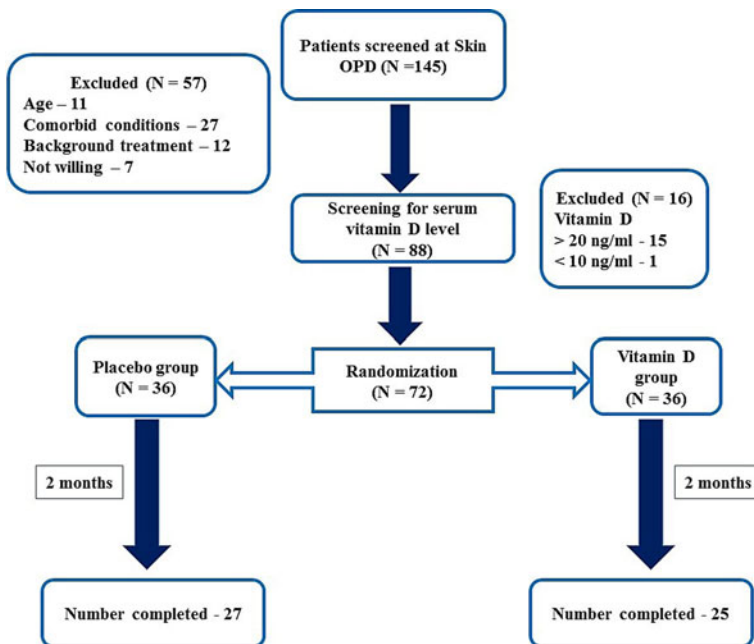


Figure 1. Participant flow diagram.

Prevalidated DLQI questionnaire (license ID CUQoL1490) was used to assess the quality of life impaired by the disease. It consists of 10 simple questions with 5 possible answers: very much, a lot, little, not at all, or not relevant. Each question can have a maximum score of 3, and the total score can range from 0 to 30 (Finlay and Khan 1994). Based on the DLQI score ranges of 0–1, 2–5, 6–10, 11–20, and 21–30, the effect on QOL can be interpreted as no effect, small effect, moderate effect, large effect, and extremely large effect, respectively.

Statistical analysis

Baseline characteristics were expressed as median and interquartile range (IQR). Proportions were described as “number with percentage.” Mann–Whitney test was used to compare baseline characteristics, serum 25-(OH)D, IL-10 levels, and change in EASI as well as DLQI scores between placebo and vitamin D groups.

Within the groups, serum 25-(OH)D and IL-10 levels at baseline and postintervention were compared by Wilcoxon signed rank test. Friedman test was used to analyze the EASI and DLQI scores across timepoints within each intervention group. Fisher’s test was used to compare the gender proportion and the frequency of adverse reactions between the two groups. $P < 0.05$ was considered statistically significant. Statistical analyses were done using SPSS, IBM version 19.0, and GraphPad Instat version 3.0.

Results

Seventy-two eligible patient participants were randomized to receive either an oral tablet of cholecalciferol 60,000 IU per week for 8 weeks ($n = 36$) or matching placebo ($n = 36$)

Table 1. Baseline demographic characteristics of study participants.

		Trial participants (N = 72)	
	Characteristic	Placebo group (n = 36)	Vitamin D group (n = 36)
1	Age (years)	57 (49.2, 59.7)	54 (45, 58)
2	Gender (male, %)	29 (81)	35 (97)
3	Weight (Kg)	56.5 (47, 60)	54.5 (48, 59)
4	BMI (Kg/m ²)	21.5 (19.2, 23.1)	21.2 (19.3, 22.8)
5	Sun exposure (in hours)	2 (1, 4)	2 (1, 3)
6	Disease duration (years)	2 (1, 3)	2.25 (1, 5)
7	EASI	15.2 (11.5, 17.4)	14.5 (11.1, 18)
8	DLQI	12 (11, 14.75)	13 (10, 15)
9	IL-10 (pg/ml)	6.4 (4.1, 11.1)	6.4 (4.1, 10.1)
10	Serum 25-(OH)D (ng/ml)	17.9 (15.4, 19.4)	16.5 (13.6, 18.2)

Values expressed as median (interquartile range); gender expressed as male *n* (%). BMI, body mass index; EASI, Eczema Area Severity Index; DLQI, Dermatology Life Quality Index; IL-10, interleukin 10; Serum 25-(OH)D, serum 25-hydroxyvitamin D.

for the same duration, in addition to the standard background treatment (Figure 1). Among them, 52 participants completed the trial.

The baseline characteristics were not significantly different between cholecalciferol and placebo groups (Table 1). The proportion of male gender was higher in the cholecalciferol group (97% versus 81%) but was not significantly different from the placebo group.

After the intervention, a significant increase was observed in 25-hydroxyvitamin D and IL-10 levels in both the groups (Table 2). The increase in serum 25-(OH)D level from baseline was significantly higher in the vitamin D group as expected. Even though the increase in IL-10 level was higher in the vitamin D group [7.7 (4.7, 8.3) pg/ml], the rise was not statistically significant compared to the placebo group.

Significant reductions in disease severity scale (EASI) and life quality index were noted after 1 month and 2 months from baseline within both intervention groups (Table 3). But the reductions in these scales were not significantly different between the two intervention groups. The observed frequency of adverse reactions reported by the study participants was not different between the groups (Table 4). The proportions of individuals with different levels of severity and quality of life grading are described (Table 5).

Discussion

Allergic contact dermatitis caused by *Parthenium* weed has been reported to be common in middle and older age groups with a male-to-female ratio ranging from 5.5:1 to 20:1 (Lakshmi and Srinivas 2007). In our study, the median age of trial participants was 56 yrs, ranging from 27 to 60 yrs, and more than 80% of them were males, with median disease duration of 2 yrs. Increased risk of exposure, dressing habits, and increased sensitivity to an allergen are the possible reasons cited for the male preponderance (Lakshmi and Srinivas 2012). *Parthenium hysterophorus* has been considered as the most common occupational allergen among farmers (Bhatia et al. 2015). Among the 72 trial participants, 43 (around 60%) were agricultural workers, who had a higher chance to get exposed to allergens at their working places.

In our study, disease severity was assessed by EASI scoring; 83.3% of study participants had moderate severity (score between 7.1 and 21), and 15.3% of patients had a

Table 2. Change in 25-hydroxyvitamin D and IL-10 levels after intervention period.

Parameter		Overall (N = 52)	Placebo group (n = 27)	Vitamin D group (n = 25)
Serum 25-(OH)D (ng/ml)	At baseline	17.6 (14.3, 18.6)	17.7 (14.7, 19.2)	16.5 (13.7, 18.2)
	After 2 months*	24.8 (19.4, 33.3) [#]	19.8 (16.0, 24.8) [#]	32.7 (25.1, 40.1) [#]
	Change*	8 (2.1, 15.8)	2.8 (0.6, 7.1)	15.3 (9.9, 23.3)
IL-10 (pg/ml)	At baseline	6.1 (4, 10.1)	6 (3.6, 8.6)	6.7 (4.1, 10.4)
	After 2 months	13.7 (10.2, 15.7) [#]	13.2 (9.7, 15.2) [#]	14.7 (12.2, 16.9) [#]
	Change	6.7 (3.9, 10.2)	5.9 (3.5, 10.6)	7.7 (4.7, 8.3)

IL-10, interleukin 10; Serum 25-(OH)D, serum 25-hydroxyvitamin D.

* $P < 0.05$, Mann–Whitney test, comparison of medians between two groups.

[#] $P < 0.05$, Wilcoxon signed rank test, Vitamin D and IL-10 levels at baseline and after 2 months among all completed trial participants.

Table 3. Effect of cholecalciferol supplementation on treatment response and quality of life.

Parameter		Overall (N = 52)	Placebo group (n = 27)	Vitamin D group (n = 25)
EASI	At baseline	15.3 (11.4, 18.4)	15.3 (11.4, 17.6)	14.4 (11.3, 20.7)
	After 1 month	12.8 (9.4, 15.0) [#]	12.8 (9, 15.3) [#]	12.7 (9.5, 14.9) [#]
	After 2 months	10.5 (9, 13.1) [#]	10.8 (8.6, 13.8) [#]	10.2 (9.2, 12.5) [#]
	Change after 1 month	−2.8 (−5.1, −0.3)	−2.4 (−4.8, 0.2)	−3.5 (−5.2, −0.2)
	Change after 2 months	−4.5 (−8.4, −0.5)	−4.4 (−8.2, −0.7)	−5.1 (−8.5, −0.4)
DLQI	At baseline	12.5 (11, 15)	12 (11, 15)	13 (11, 15)
	After 1 month	11 (9, 14) [#]	11 (9, 14) [#]	11 (8, 13.5) [#]
	After 2 months	10 (8, 12) [#]	10 (8, 12) [#]	10 (6.5, 11.5) [#]
	Change after 1 month	−1.5 (−4, 0)	−2 (−3, 0)	−1 (−4.5, −0.5)
	Change after 2 months	−3 (−5, −1)	−3 (−5, −1)	−4 (−7, −1)

EASI, Eczema Area Severity Index; DLQI, Dermatology Life Quality Index.

[#] $P < 0.05$, Friedman test, EASI and DLQI scores at baseline, after 1 month and 2 months among all completed trial participants in both intervention groups.

Table 4. Adverse effects reported among trial participants.

Symptom		Number reported (overall)	Placebo	Vitamin D
1	Nausea	5	2	3
2	Abdominal discomfort	8	4	4
3	Abdominal pain	9	5	4
4	Tiredness	11	7	4
5	Loss of appetite	8	3	5
6	Dry mouth	7	4	3
7	Sleeplessness	9	4	5
8	Others	6	3	3

Table 5. Disease severity and quality-of-life grading at baseline and after 2 months of intervention among study participants.

		Placebo (n = 27)		Vitamin D (n = 25)	
Grade	Baseline (overall, N = 72)	Baseline	After 2 months	Baseline	After 2 months
Disease severity grade (EASI)					
Mild	1 (1.4)	0	5 (19)	1 (4)	2 (8)
Moderate	60 (83.3)	23 (85)	22 (81)	18 (72)	23 (92)
Severe	11 (15.3)	4 (15)	0	6 (24)	0
Quality-of-life grade (DLQI)					
Small	0	0	2 (7.4)	0	5 (20)
Moderate	16 (22)	4 (15)	12 (44.4)	5 (20)	10 (40)
Large	56 (78)	23 (85)	13 (48.1)	20 (80)	10 (40)

Values expressed as n (%). EASI, Eczema Area Severity Index; DLQI, Dermatology Life Quality Index.

severe grade (score between 21.1 and 50). The median DLQI score was 12 with the interquartile range between 11 and 15. A majority of the participants (56 out of 72; 78%) had experienced a large effect on their quality of life because of the disease

condition. In a previous study, the effect of occupational contact dermatitis on quality of life was graded as very large in more than 60% of the patients (Bhatia et al. 2015).

The median plasma IL-10 level among trial participants was 6.4 (4.1, 10.4) pg/ml [median (IQR)]. IL-10 levels observed among the *Parthenium* dermatitis patients were significantly lower compared to the level observed among healthy controls (9 pg/ml) in our lab. The results were similar to the observations made in scientific literature. Significantly lower levels of Th2 cytokine IL-10 were observed among patients with *Parthenium*-induced contact dermatitis (4.2 versus 7.9 pg/ml in controls; Akhtar et al. 2010a, 2010b). Khatri and colleagues noted the mean IL-10 level of 4.2 pg/ml among patients compared to 8.2 pg/ml among healthy controls (Khatri et al. 2011). IL-10 being the important anti-inflammatory cytokine, inadequate levels could result in inefficient control of inflammation, and the manifestations follow thereafter.

Among the *Parthenium* patients screened, 83% had serum 25-(OH)D levels less than 20 ng/ml, which is considered deficient per Endocrine Society guidelines (Holick et al. 2011). Decreased sun exposure to prevent disease exacerbation and decreased capability of the diseased skin to synthesize vitamin D from sunlight could be the possible reasons for the increased prevalence of vitamin D deficiency among contact dermatitis patients.

The patients attending the dermatitis clinic for disease exacerbation were treated with oral as well as topical steroids for initial disease control. Antihistamines and barrier creams were given for symptomatic treatment. Systemic steroids were tapered gradually, and oral azathioprine (50 mg once a day or twice daily) was started after ensuring normal liver function, checking biochemical parameters, and ruling out the risk factors for using immunosuppressants. The patients were instructed to follow preventive measures to avoid exposure to allergens to reduce the risk of exacerbation. Among 72 participants recruited, 20 (28%) were lost to follow-up. Economic reasons that prevent a regular visit to the hospital and chronic course of the disease requiring continuous treatment were the common reasons noted for loss to follow-up. With the achieved sample size, the final power of the study was calculated to be around 80%.

The literature evidence to support the benefit of correcting serum 25-(OH)D level in allergic contact dermatitis is scarce (Quirk et al. 2016); preclinical studies suggest that vitamin D could reduce cutaneous inflammation (Malley et al. 2009, 2013). *Parthenium* dermatitis patients were observed with dysregulated Th2 cytokines, including decreased IL-10 levels and increased frequency of low IL-10-producing genotypes. The ability of IL-10 to reduce the effector phase of contact sensitivity is also appreciated in the literature, prompting us to search the evidence regarding improving IL-10 levels in *Parthenium* dermatitis patients (Asadullah et al. 2003).

The ability of 1,25-dihydroxy vitamin D₃ to increase IL-10 production and expression has been documented by preclinical and clinical studies in the literature (Ashtari et al. 2015; Bakdash et al. 2014; Cantorna et al. 2015; Farsani et al. 2015; Matilainen et al. 2010; Schleithoff et al. 2006; Sommer and Fabri 2015). The dose and duration of cholecalciferol supplementation used in our study is the most common regimen followed in India by physicians. In a study done in Asian Indians, the 8-week regimen with 60,000 IU per week was found to be effective to correct serum 25-(OH)D status, but to maintain the levels at sufficient range for 1 yr, continuous supplementation was required. At the end of 8 weeks supplementation the mean serum 25-(OH)D increased

from 5.4 ± 1.2 ng/ml to 32.96 ± 8.28 ng/ml, which was reduced to 9.88 ± 4.36 ng/ml at the end of 12 months (Goswami et al. 2008). In our study after the intervention period, significant improvement in 25-(OH)D level was seen in both placebo and cholecalciferol groups, but the rise in serum 25-(OH)D level was significantly greater in the cholecalciferol group, from 16.5 (13.7, 18.2) ng/ml [median (IQR)] to 32.7 (25.1, 40.1) ng/ml. The increased serum 25-(OH)D level in placebo group could be due to the improvement in skin condition and diet and lifestyle changes among study participants after knowing about the vitamin's importance.

After supplementation, serum 25-(OH)D levels were raised to more than 20 ng/ml in 92% of the patients in the cholecalciferol group, compared to 48% in placebo group; 60% of the individuals (15 of 25) in the active intervention group had serum 25-(OH)D levels of more than 30 ng/ml after 2 weeks, and 32% of the individuals (8 out of 25) had levels between 20 ng/ml and 20.9 ng/ml. Changes in IL-10, EASI score, and DLQI were compared between individuals who achieved serum 25-(OH)D more than 30 ng/ml and individuals with levels 20–29.9 ng/ml after cholecalciferol supplementation. No significant differences between the groups were observed for the three parameters.

The rise in IL-10 observed in the vitamin D group was higher than in the placebo group (7.7 versus 5.9), but the change was not statistically different between the groups. In the vitamin D group 23 people out of 25 had IL-10 level above 9 pg/ml after supplementation, compared to 24 people out of 27 in the placebo group, with the odds ratio of 1.44 (95% CI [0.22, 9.41], $p = 0.70$). Apart from vitamin D supplementation, the background treatment with oral azathioprine and clinical improvement in disease condition also could have contributed to the rise in IL-10 levels. Isolated T cells and macrophages from Crohn's disease patients treated with azathioprine showed about 10-fold increase in IL-10 levels. It was also proposed that the effect of azathioprine on modulating inflammation could be mediated through IL-10 (Fuss et al. 1999). Since the background treatment was similar in the two groups, the higher change in IL-10 from baseline could be because of cholecalciferol supplementation.

Administration of cholecalciferol in a dose of 2,000 IU per day for 3 months did not show a significant change in cytokine levels, including IL-10, compared to placebo in healthy adults. But the mean serum 25-(OH)D levels were increased from 25.72 ± 10.16 ng/ml to 35.4 ± 9.28 ng/ml in the active intervention group (Yusupov et al. 2010). In a randomized placebo-controlled trial done in congestive heart failure patients, 43% increase in the median IL-10 level was seen in the cholecalciferol group (2,000 IU with 500 mg calcium per day) after 9 months compared to baseline. A significant change in serum 25-(OH)D level of 26.8 (9.2, 35.1) ng/ml [median (IQR)] from the baseline value of 14.4 (11.5, 22.1) ng/ml was noted (Schleithoff et al. 2006). Cholecalciferol administration (50,000 IU per week for 8 weeks) was reported to increase IL-10 expression 3.84 times in vitamin D deficient multiple sclerosis patients. In addition, the serum 25-(OH)D levels increased significantly ($p < 0.0001$) from 12.19 ± 5.88 ng/ml at baseline to 42.66 ± 17.35 ng/ml after supplementation (Farsani et al. 2015).

In another study, different dose regimens of cholecalciferol supplementation did not have a significant effect on peripheral circulating cytokines in apparently healthy adults. Sufficient serum 25-(OH)D levels among the volunteers at baseline with median levels of 28.4 ng/ml and 22.12 ng/ml in the younger and older cohorts, respectively, were

suggested to be a possible reason for the lack of change (Barnes et al. 2011). Failure to demonstrate the role of cholecalciferol supplementation on the IL-10 level in a healthy population could be because the immune functions are already in balance. In disease conditions where immune homeostasis is at stake, cholecalciferol may be helping to attain the balance, possibly by increasing anti-inflammatory cytokine IL-10.

A meta-analysis was done to find out the relationship between serum 25-(OH)D levels and inflammatory markers; it included around 39 randomized controlled trials (RCTs) with positive, negative, and mixed outcomes. In RCTs studying cholecalciferol supplementation as an active intervention, baseline serum 25-(OH)D less than 18 ng/ml and postintervention levels between 34 and 40 ng/ml were the important factors that could help prove the effect of vitamin D on inflammatory markers. High inflammatory conditions with elevated inflammatory markers at baseline were the other factors determining the study outcome (Cannell et al. 2014). In our study the baseline median serum 25-(OH)D level was 16.5 ng/ml, and postintervention it was raised to 32.7 ng/ml. This lower increase in levels could be a contributory factor in not finding significant change in IL-10 levels.

In both groups a decreasing trend was observed in EASI and DLQI scores from baseline after 1 month and 2 months of intervention. The reduction in EASI severity score was 3.5 versus 2.4 (cholecalciferol versus placebo) at 1 month and 5.1 versus 4.4 after 2 months of the intervention period. Reduced difference between two groups was observed possibly because of active background treatment and the chronic nature of the disease. Extending the duration of follow-up further could have helped us to appreciate the difference between two groups more clearly.

Out of 52 participants who had completed the intervention, only 27 completed telephone follow-up for 4 months. Among them, 3 patients experienced relapse with worsening of symptoms and required oral steroid treatment. One of them belonged to the cholecalciferol group and 2 to the placebo group. One of the patients who relapsed in the placebo group had discontinued treatment for 1 month during the follow-up period.

Adherence to cholecalciferol or placebo was calculated based on the refill of the sealed envelope received by the participants from the investigator. The overall adherence for intervention was 84%, with 87% and 80% in placebo and cholecalciferol groups, respectively. The adherence to azathioprine was calculated considering the medication refill the patients received from the hospital pharmacy. The adherence to azathioprine among participants in placebo and cholecalciferol groups was 91% and 87%, respectively, and the overall adherence observed for azathioprine was 89%.

The adverse reactions to prescribed medications and administered intervention were noted in a predesigned checklist containing possible adverse drug reactions (ADRs). Gastrointestinal symptoms were the most commonly observed ADRs among the completed trial participants. The ADR frequency was not different between the two intervention groups.

Reduced IL-10 levels in *Parthenium* dermatitis patients and increased posttreatment levels suggest a possible role of IL-10 therapy in the management. Even though our study could not find a statistically significant difference with cholecalciferol supplementation, it has generated evidence to support its role in reducing disease severity and improving IL-10 levels. Measuring both inflammatory and anti-inflammatory markers,

their expression levels, and serum 25-(OH)D levels in contact dermatitis patients will help in elucidating their role in pathophysiology and the sequelae of bringing balance among them.

Conclusion

Cholecalciferol supplementation did not significantly reduce the disease severity or improve the quality of life in *Parthenium* dermatitis patients compared to placebo. Although a decreasing trend in severity was noted in both groups, the cholecalciferol group had a slightly better reduction. Treatment for 2 months significantly improved the plasma IL-10 levels in both placebo and cholecalciferol groups, with a higher increase in the latter.

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Declaration of interest

The authors declare no conflicts of interest. The authors alone are responsible for the content and writing of the article.

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