Type of the Paper (Research Article)

Evaluation of serum vitamin D3 before and after intralesional vitamin d3 injection in patients with alopecia areata

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Received: 17 August, 2024 Reviewed: 1 December, 2024

Accepted: 25 January, 2025 Published online: 6 November, 2025

Abstract

This study investigates the effect of intralesional vitamin D3 injections on serum 25-hydroxyvitamin D3 levels in patients with localized patchy alopecia areata. Twenty adults received three intralesional sessions over nine weeks. Results showed a significant increase in serum vitamin D3 levels from 9.31 ng/mL to 23.55 ng/mL ($p = 0.001$). The proportion of deficient patients dropped from 85% to 30%, while those with optimal levels rose from 10% to 35% ($p = 0.012$). Despite these improvements, no significant correlation was observed between baseline vitamin D3 levels and the severity of alopecia areata (rho = 0.368, $p = 0.110$). The findings suggest that intralesional vitamin D3 is not only effective in improving systemic vitamin D3 status but also offers therapeutic potential for alopecia areata, although baseline serum levels are not predictive of disease severity or treatment response.

Keywords: Alopecia areata ،Intralesional vitamin D3، Serum 25(OH)D, Hair loss therapy, Autoimmune skin disorder

Aim of study: This study aimed to evaluate the changes in serum vitamin D3 levels following intralesional administration of vitamin D3 in patients diagnosed with patchy alopecia areata. By assessing vitamin D3 levels both before and after treatment, the study sought to determine whether local administration of vitamin D3 has a systemic effect on circulating vitamin D levels. The research also aimed to observe if the serum level improvements correspond with clinical outcomes, potentially offering insights into vitamin D3's systemic absorption and therapeutic role beyond local site effects. This interventional trial involved twenty adult patients who received three sessions of intralesional vitamin D3 injections. The serum vitamin D3 levels were quantitatively measured at baseline and after the final injection. The study contributes to understanding the systemic implications of localized vitamin D3 therapy and its potential utility in managing alopecia areata.

1. Introduction

Therapeutically, vitamin D analogues have been explored in various formulations. While topical calcipotriol has shown limited but promising effects [9], the intralesional administration of cholecalciferol represents a novel method with direct follicular targeting, enhanced bioavailability, and minimized systemic exposure . Intralesional therapy may also overcome the poor skin penetration limitations of topical treatments, offering an alternative for patients unresponsive to corticosteroid immunosuppressants [10, 11] .

Captivatingly, while the local effects of intralesional vitamin D3 on hair regrowth have been observed, its systemic impact on serum 25(OH)D levels has received little attention. Understanding whether localized intradermal injections can restore or elevate serum vitamin D levels could have broader implications, especially in populations where vitamin (D) deficiency is endemic [12, 13].Moreover, the relationship between baseline vitamin D levels and clinical responsiveness to such therapy stay put controversial, with some studies indicating no predictive value .Given these gaps, our study aims to evaluate serum vitamin D3 levels before and after intralesional administration of cholecalciferol in patients with patchy Alopecia areata, and to explore whether such intervention can modify systemic vitamin D status in addition to promoting localized hair regrowth.

2. Subjects and methods

- Adults aged 18 to 55 years with clinically confirmed localized patchy alopecia areata.

Alopecia areata is a recurrent, autoimmune, non-scarring form of hair loss that targets anagen hair follicles, resulting in sudden, patchy baldness predominantly on the scalp and sometimes other body areas. It affects about 0.1–0.2% of the population, with an increasing burden in young adults and females in recent years [1]. The psychological and cosmetic implications of Alopecia areata are profound, often leading to depression, anxiety, and poor self-image [2].Recent advances have highlighted the role of immune dysregulation in Alopecia areata pathogenesis, especially involving CD8+ T cells, loss of hair follicle immune privilege, and upregulation of interferon-gamma pathways [3]. One of the most promising immunomodulatory agents in this context is vitamin D3, which has been shown to exert direct regulatory effects on both the innate and adaptive immune systems [4].

Vitamin D receptors (VDR) are expressed in hair follicle keratinocytes, and animal models deficient in VDR exhibit impaired folliculogenesis and alopecia [5].Several studies since 2017 have consistently shown that serum 25-hydroxyvitamin D levels are expressively lower in patients with Alopecia areata compared to healthy individuals [6]. However, the clinical significance of this association whether vitamin D deficiency contributes to Alopecia areata onset or is a restrained consequence of autoimmune dysregulation remains under debate [7, 8].

2.1. Subjects

Inclusion Criteria:

minimize confounding factors that may influence treatment response or serum vitamin D3 levels.

2.2. Study design

1. Study Design and Population

This interventional therapeutic study was designed as a prospective single-arm clinical trial aimed at evaluating the systemic effect of intralesional vitamin D3 administration on serum vitamin D3 levels in patients with alopecia areata. The study enlisted twenty adult patients, all diagnosed with localized patchy alopecia areata based on clinical examination by a dermatologist. Inclusion criteria required participants to be between the ages of 18 and 50 years, in good general health, and free from any systemic or autoimmune diseases that could influence vitamin D metabolism, such as systemic lupus erythematosus, diabetes mellitus, or inflammatory bowel diseases.

To ensure uniformity, only patients who had not received any treatment for alopecia areata either topical or systemic during the three months prior to the study enrollment were included. Patients were excluded if they were currently on vitamin D supplementation, had a known hypersensitivity to vitamin D3, were pregnant or breastfeeding, or had any hepatic or renal impairment that could affect vitamin D3 metabolism. Prior to enrollment, written informed consent was

- Patients with at least one stable alopecic patch persisting for more than 3 months.
- Willingness to undergo intralesional vitamin D3 treatment and provide informed consent.
- Baseline serum 25-hydroxyvitamin D levels below 30 ng/mL (i.e., deficient or insufficient status).
- No prior treatment for alopecia areata in the preceding 6 weeks.

Exclusion Criteria:

- Patients with diffuse or total alopecia (alopecia totalis or universalis).
- Individuals with underlying scalp infections, dermatitis, or other dermatologic conditions.
- History of hypersensitivity to vitamin D3 or its derivatives.
- Concurrent uses of systemic immunosuppressive or corticosteroid therapies.
- Pregnant or breastfeeding women.
- Patients with systemic illnesses affecting vitamin D metabolism (e.g., sarcoidosis, hyperparathyroidism, chronic kidney disease).
- Serum calcium level outside the normal reference range.
- Participation in another clinical study within the last 3 months**.**

These criteria were implemented to ensure a homogenous study population and

topical anesthetic cream (lidocaine 2.5% and prilocaine 2.5%) applied under occlusion for 30 minutes to minimize patient discomfort. Injections were played under sterile conditions in an outpatient dermatology clinic by a trained physician.

Throughout the intervention period, patients were monitored for any adverse effects, such as local pain, swelling, erythema, or systemic symptoms. A standardized checklist was used to document side effects immediately after the procedure and during follow-up visits.

3. Laboratory Assessment

The evaluation of serum vitamin D3 levels was conducted through blood samples collected twice during the study: the first sample was taken at baseline (prior to the initial injection), and the second sample was obtained three weeks after the final intralesional session (at week 9). Blood samples were drawn from the antecubital vein using a sterile vacutainer system and were processed within 1 hour of collection.

Serum 25-hydroxyvitamin D [25(OH)D], the most reliable biomarker for vitamin D status, was quantified using standardized chemiluminescence immunoassay (CLIA), which is known for its high specificity and sensitivity. All laboratory procedures were managed in a certified clinical laboratory, and the personnel were blinded to the clinical data to avoid measurement bias. Vitamin D3 levels were classified into three categories based on the Endocrine Society guidelines:

obtained from all participants, and the study protocol was approved by the local ethics committee in accordance with the Declaration of Helsinki.

Demographic data such as age, sex, disease onset, duration of lesions, family history, and history of autoimmune diseases were recorded at baseline. This documentation safeguarded proper patient characterization and allowed for secondary subgroup analyses if necessary.

2. Intervention Protocol

The therapeutic intervention consisted of intralesional injections of vitamin D3, specifically in the form of an aqueous cholecalciferol solution. Each ampoule contained 100,000 IU of cholecalciferol dissolved in 2 ml of sterile aqueous solution. The preparation was selected for its known pharmacokinetics, safety profile, and previous evidence supporting its use in dermatological conditions.

Patients received injections once every three weeks, for a total of three sessions over approximately nine weeks. Each injection was administered directly into the alopecic patches using a fine insulin syringe, and care was taken to disperse the dose evenly across the lesion area. The maximum volume per session did not exceed 1 ml, corresponding to 50,000 IU of vitamin D3 per treated site.

Prior to the injections, the affected area was cleaned and anesthetized using a

two response categories. This provided insights into the clinical impact of the intervention in terms of actual improvement in vitamin D status, not just numerical increase.

Secondary observations included exploring any correlation between baseline vitamin D levels and the severity of alopecia areata, which was measured using a clinical severity index. Although not the main focus, this helped assess whether serum vitamin D status had any predictive value for disease severity or response to treatment. Adverse events were monitored throughout the study and documented during each follow-up session. These included local reactions such as pain at the injection site, erythema, induration, or systemic symptoms like fatigue or dizziness. The safety profile was considered an essential secondary endpoint to evaluate the tolerability of intralesional vitamin D3 in a clinical setting.

- Deficient: <20 ng/mL
- Insufficient: 20–29 ng/mL
- Sufficient (optimal): ≥30 ng/mL

These categories were used to interpret changes in vitamin D3 status pre- and post-intervention and assess the proportion of patients achieving optimal serum levels after treatment.

4. Outcome Measurement

The primary outcome of the study was the change in serum vitamin D3 levels following the intralesional administration of cholecalciferol. To evaluate this, paired comparisons of baseline and post-treatment serum 25(OH)D levels were conducted. The Wilcoxon signed-rank test was employed to assess the significance of the median difference in continuous vitamin D3 levels due to the non-parametric distribution of the data.

In addition, categorical shifts in vitamin D status (from deficient to insufficient or sufficient) were analyzed using the McNemar-Bowker test, which is suitable for paired nominal data with more than

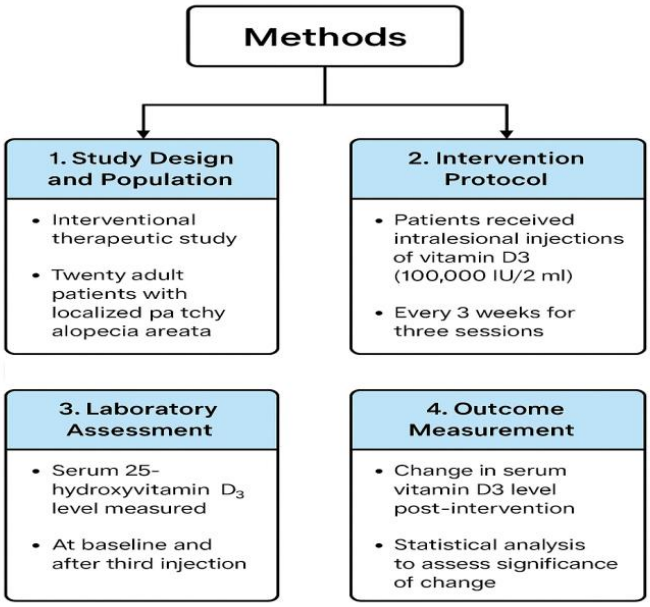

Figure 1: Overview of Study Methods for Evaluating Serum Vitamin D3 Levels in Alopecia Areata Patients

3. Results:

while those achieving optimal levels rose from 10% to 35%, according to McNemar-Bowker test (p = 0.012). Despite these improvements, no significant correlation was found between baseline vitamin D3 levels and alopecia severity, as assessed by the Alopecia Areata Severity Index (AASI). These findings suggest that while intralesional vitamin D3 effectively improves systemic vitamin D status, it may not directly predict disease severity.

3.1 | *Change in Median Serum Vitamin D3 Levels*

Following the administration of intralesional vitamin D3, a statistically significant increase in serum 25-hydroxyvitamin D3 levels was observed among the study participants. The median concentration rose from 9.31 ng/mL before treatment to 23.55 ng/mL after completing three injections, with a p-value of 0.001 according to the Wilcoxon signed-rank

4. This interventional therapeutic study explored the impact of intralesional vitamin D3 (cholecalciferol) injections on serum 25-hydroxyvitamin D3 levels in 20 adult patients with clinically diagnosed localized patchy alopecia areata. Each patient received three sessions of intralesional vitamin D3 over a nine-week period, with serum samples collected both before the first injection and three weeks after the final session. The aim was to evaluate whether localized vitamin D3 therapy could produce systemic changes in serum levels.

A statistically significant increase in serum vitamin D3 was recorded, with median levels rising from 9.31 ng/mL pre-treatment to 23.55 ng/mL post-treatment (p = 0.001). Additionally, the proportion of patients with vitamin D3 deficiency decreased markedly from 85% to 30%,

9.31 ng/mL, confirming a state of deficiency in most patients, while the right bar shows a substantial increase to 23.55 ng/mL following treatment. The presence of the asterisk and p-value (0.001) highlights the statistical significance of this change. This result supports the efficacy of intralesional vitamin D3 in correcting vitamin D3 deficiency and reinforces its potential role as both a local and systemic therapeutic agent in patients with alopecia areata.

test. This significant elevation suggests that vitamin D3, when delivered intralesional, is not only effective locally but also capable of being systemically absorbed, leading to measurable biochemical improvements.

As demonstrated in Figure 2, the bar chart provides a clear visual comparison of serum vitamin D3 levels before and after the intervention. The left bar indicates the baseline median level of

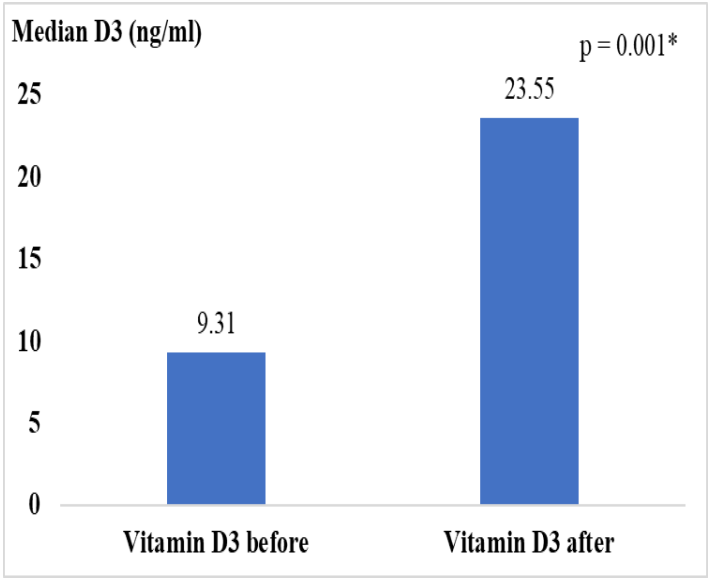

Figure 2. Serum Vitamin D3 Level Before and After Intralesional Injection. (Wilcoxon signed-rank test, p = 0.001)

meaningful improvement in patient classification.

Before intervention, the vast majority of participants (17 out of 20, representing 85%) were classified as deficient, while only 1 patient (5%) fell into the insufficient range, and just 2 patients (10%) had optimum serum levels.

After completing three intralesional sessions, a remarkable redistribution occurred:

- 3.2 | *Categorical Shift in Vitamin D3 Status*

Following the administration of intralesional vitamin D3, there was a notable shift in the distribution of patients across the categories of vitamin D3 status. These categories are defined as deficient (<20 ng/mL), insufficient (20–29 ng/mL), and optimum (≥30 ng/mL). The therapeutic impact of the intervention extended beyond numerical increases in serum concentration to a clinically

Statistical validation using the McNemar–Bowker test confirmed the significance of this shift, yielding a p-value of 0.012, denoting a strong statistical association between intralesional vitamin D3 and improved serum classification.

Further support for this finding is provided in Table 1, which summarizes the patient counts and percentages across both time points:

This redistribution emphasizes the dual benefit of intralesional therapy: not only did it increase serum vitamin D3 concentrations, but it also moved a majority of patients toward more favorable clinical status.

- The deficient group decreased substantially to 6 patients (30%).
- The insufficient group increased to 7 patients (35%).
- Importantly, the number of patients achieving optimum vitamin D3 status rose to 7 (35%).

This shift is clearly depicted in Figure 3, a stacked column chart that contrasts the percentage distribution before and after the intervention. Each column is segmented by status category, with blue for deficient, orange for insufficient, and grey for optimum. The visual difference between the two columns vividly illustrates the therapeutic effect: a clear reduction in deficiency and a corresponding increase in sufficiency.

Table 1. Serum Vitamin D3 levels (categories) before and after the intervention.

	Vitamin D3 before intervention	Vitamin D3 after intervention	
D3 levels	No. (%)	No. (%)	p-value*
Deficient	17 (85.0)	6 (30.0)	
Insufficient	1 (5.0)	7 (35.0)	0.012
Optimum	2 (10.0)	7 (35.0)	
Total	20 (100.0)	20 (200.0)	

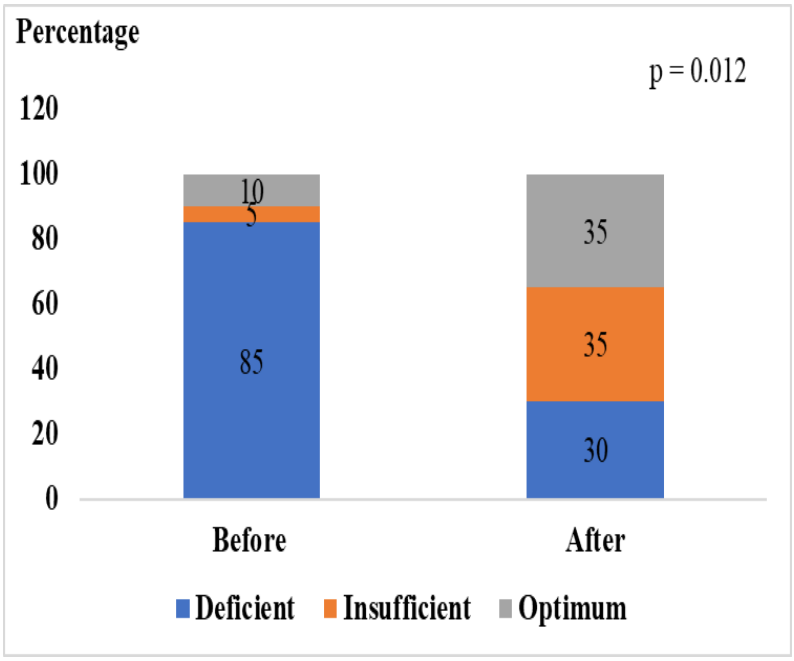

Figure 3. Serum Vitamin D3 levels (categories) before and after the intervention

standard threshold of 0.05 for statistical significance.

This means that although there is a mild upward trend, suggesting that higher vitamin D levels might be loosely associated with higher AASI scores, the variation is not sufficient to establish a meaningful or predictive relationship. Hence, the data does not support a conclusion that initial vitamin D status influences or predicts alopecia severity.

In summary, Figure 4 clearly demonstrates the absence of a significant correlation, reinforcing the notion that while vitamin D3 supplementation improves biochemical status, it may not directly reflect the clinical pattern or progression of alopecia areata.

3.3 | *Correlation with Clinical Severity of Alopecia*

Although intralesional vitamin D3 significantly improved serum vitamin D3 levels, the relationship between baseline vitamin D3 levels and disease severity was not statistically significant. The clinical severity of alopecia was assessed using the Alopecia Areata Severity Index (AASI), which quantifies the extent of hair loss.

As depicted in Figure 3, the scatterplot shows the distribution of patients according to their serum vitamin D levels (x-axis) and corresponding AASI scores (y-axis). A trendline was fitted to the data, and while a slight positive slope is observed, indicating a weak association, the correlation coefficient (rho) is 0.368 with a p-value of 0.110, which exceeds the

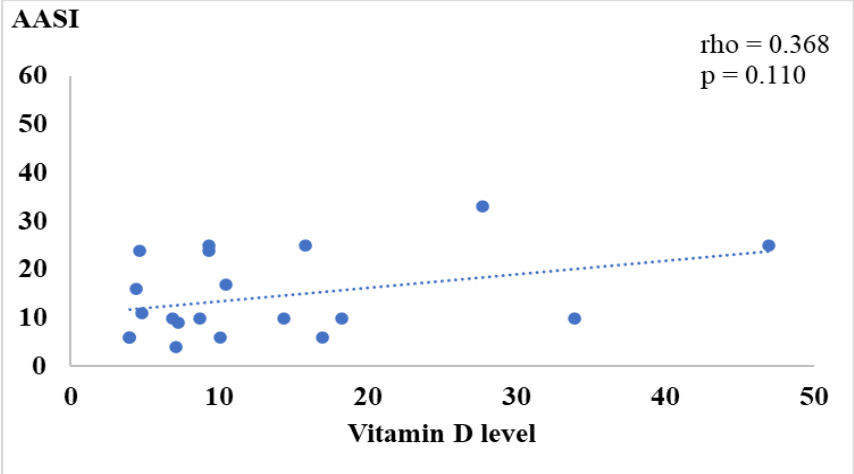

Figure 4. Scatterplot Depicting the Lack of Correlation Between Baseline Serum D3 and Alopecia Severity Score (AASI)

marker for disease severity in clinical assessments.

3.4 | *Interpretation of Findings*

The findings of this study provide strong evidence that intralesional vitamin D3 injections are effective in significantly increasing serum 25-hydroxyvitamin D3 levels in patients with localized alopecia areata. Prior to treatment, 85% of the patients (17 out of 20) were categorized as vitamin D3 deficient (serum levels < 20 ng/mL), while only 10% (2 patients) had optimum levels (≥30 ng/mL). Following three intralesional sessions over nine weeks, the proportion of deficient patients dropped sharply to 30% (6 patients). Simultaneously, the percentage of patients reaching optimum vitamin D3 levels increased from 10% to 35%, with 7 patients achieving sufficient levels. The shift in vitamin D3 categories was statistically significant ($p = 0.012$, McNemar-Bowker test), demonstrating the systemic benefit of the localized treatment.

Moreover, the median serum vitamin D3 concentration rose from 9.31 ng/mL before treatment to 23.55 ng/mL after treatment, a statistically significant

This figure 4 illustrates the relationship between baseline serum vitamin D (25-hydroxyvitamin D) levels and the clinical severity of alopecia areata, as measured by the Alopecia Areata Severity Index (AASI). Each dot represents an individual patient (n = 20). The x-axis indicates the serum vitamin D level in ng/mL, while the y-axis reflects the corresponding AASI score, which quantifies the extent of hair loss.

A trendline has been added to represent the general direction of association. The Spearman correlation coefficient (rho) is 0.368, suggesting a weak positive correlation; however, the associated p-value is 0.110, indicating that the correlation is not statistically significant at the conventional alpha level ($p < 0.05$). This means that although a slight increase in AASI score appears to accompany higher vitamin D levels, the relationship lacks sufficient evidence to be deemed meaningful in a statistical sense. the figure 4 demonstrates that baseline serum vitamin D levels do not significantly correlate with the severity of alopecia areata, reinforcing the conclusion that serum vitamin D status alone should not be used as a predictive

scatterplot analysis revealed a weak correlation (rho = 0.368) with a p-value of 0.110, indicating the relationship was not statistically significant.

Therefore, while intralesional vitamin D3 is promising for enhancing serum levels and potentially improving clinical outcomes, the baseline vitamin D status alone cannot be relied upon as a predictive marker for disease severity or treatment response in alopecia areata

4. Discussion

However, the absence of a significant correlation between baseline vitamin D3 levels and alopecia severity (rho = 0.368, p = 0.110) suggests that serum vitamin D may not be a reliable predictor of disease burden or response to treatment. This finding is consistent with previous reports indicating that while vitamin D deficiency is common in alopecia areata, its role in disease progression remains unclear. Intralesional vitamin D3 appears to be a promising adjunctive therapy for alopecia areata with favorable systemic impact. Nevertheless, further randomized controlled trials with larger sample sizes and longer follow-up are needed to better define its predictive value and long-term efficacy.

5. Conclusion

ng/mL before treatment to 23.55 ng/mL post-treatment (p = 0.001, Wilcoxon signed-rank test). This confirms that localized delivery of vitamin D3 can lead to measurable systemic absorption.

Furthermore, a significant categorical shift was observed in vitamin D3 status. Initially, 85% of patients were deficient, while 10% had optimal levels. After

increase (p = 0.001, Wilcoxon signed-rank test). This supports the view that intralesional delivery, though targeted locally to alopecic patches, can have systemic absorption and therapeutic effects.

Despite these improvements in biochemical parameters, the study found no significant correlation between baseline serum vitamin D3 levels and the severity of alopecia, as measured by the Alopecia Areata Severity Index (AASI). The

This study provides compelling evidence that intralesional vitamin D3 therapy not only improves local alopecia areata lesions but also leads to significant systemic biochemical changes. The sharp increase in serum 25-hydroxyvitamin D3 levels from a median of 9.31 ng/mL to 23.55 ng/confirms systemic absorption following local administration. This is especially important in regions with high prevalence of vitamin D deficiency, where such treatment may offer dual benefits. Furthermore, the proportion of patients with vitamin D deficiency was reduced from 85% to 30%, and those with optimal levels increased from 10% to 35%, demonstrating clinically meaningful improvement (p = 0.012).

This interventional therapeutic study assessed the impact of intralesional vitamin D3 injections on serum 25-hydroxyvitamin D levels in 20 adult patients with localized patchy alopecia areata. Patients received three sessions of intralesional cholecalciferol (100,000 IU/2 ml) over nine weeks. Results showed a statistically significant increase in median serum vitamin D3 levels, rising from 9.31

willingly took part in this research and contributed to its success.

Special thanks to the laboratory team for their assistance in serum sample collection and vitamin D3 analysis, and to the ethical review board for their valuable oversight and approval of the study protocol.

Ethical approval and consent to participate:

The authors declare that they have not used any type of generative artificial intelligence for the writing of this manuscript, nor for the creation of images, graphics, tables, or their corresponding captions.

treatment, only 30% remained deficient, and 35% reached optimum levels. The McNemar–Bowker test validated this redistribution ($p = 0.012$), indicating a meaningful improvement in systemic vitamin D status.

However, no statistically significant correlation was found between baseline vitamin D levels and disease severity as measured by the Alopecia Areata Severity Index (AASI). A weak non-significant

Acknowledgment

The authors express their sincere gratitude to the Department of Dermatology, [Your Institution's Name], for their continuous support and for providing the clinical facilities required for this study. We would also like to thank all the participants who

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