



Original Article

Comparative Efficacy of Microneedling with and without Topical Vitamin D3 in Localized *Alopecia areata*Rameen Masood¹, Faria Asad¹, Hira Tariq¹, Uzma Amin¹ and Abrar Ul Haq¹¹Department of Dermatology, Services Hospital, Lahore, Pakistan

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ABSTRACT

Alopecia areata represents a T-cell-mediated autoimmune disorder causing non-scarring hair loss. While microneedling demonstrates therapeutic potential, adjunctive strategies to enhance efficacy remain underexplored. **Objectives:** To compare the efficacy and safety of microneedling combined with topical vitamin D3 versus microneedling monotherapy in localized *alopecia areata*. **Methods:** An open-label randomized controlled trial enrolled 80 participants (April–October 2025) with localized patchy *alopecia areata* affecting <50% scalp. Participants were randomly allocated to microneedling combined with topical vitamin D3 (MN+VD3, n=40) or microneedling alone (MN, n=40). Treatment consisted of six biweekly sessions over 12 weeks. Primary efficacy endpoints included Severity of Alopecia Tool (SALT) score reduction and percentage hair regrowth. **Results:** Mean SALT reduction was significantly greater in the MN+VD3 group (13.27 ± 4.284 points) compared with MN (9.66 ± 3.827 points, $p=0.001$). Hair regrowth percentage was superior in MN+VD3 ($65.737 \pm 18.6252\%$) versus MN ($48.137 \pm 24.0500\%$, $p<0.001$). Excellent or good response was achieved in 82.5% of MN+VD3 participants versus 50.0% of MN participants ($p=0.002$). Stratified analysis demonstrated consistent treatment superiority across age groups (18–40 and 41–60 years), both sexes, and disease duration categories. Adverse events were mild and transient, occurring in 30.0% overall with no serious complications or treatment discontinuations. **Conclusions:** Topical vitamin D3 combined with microneedling substantially enhances therapeutic outcomes compared with monotherapy, with excellent tolerability and sustained efficacy across demographic substrata. These findings support adjunctive vitamin D3 as an effective treatment strategy for localized *alopecia areata*.

INTRODUCTION

Alopecia areata is a relapsing autoimmune disorder of the hair follicle that presents with non-scarring, well-demarcated patches of hair loss on the scalp or other hair-bearing sites [1]. Global lifetime prevalence is estimated at 1% to 2.1%, while point prevalence in population-based studies is approximately 0.1% to 0.2% [2, 3]. The course is difficult to predict; some individuals have transient episodes with spontaneous regrowth, whereas others experience recurrent or persistent disease over many years [4]. Beyond cosmetic change, visible hair loss can impair social functioning and psychological well-being,

with increased anxiety and depressive symptoms reported in many patients. Given relapse tendency and the need for repeated outpatient treatment, safe and acceptable interventions that produce meaningful regrowth remain a clinical priority [5]. Pathogenesis involves loss of hair follicle immune privilege during the anagen phase in genetically susceptible individuals. This immune dysregulation permits antigen recognition and is associated with perifollicular infiltration of cytotoxic and helper T lymphocytes, alongside increased interferon-gamma, interleukin-15, and other inflammatory mediators



[6, 7]. Vitamin D receptor signalling in keratinocytes and hair follicle cells influences hair cycle dynamics and cutaneous immune responses, and reduced vitamin D status has been reported frequently in individuals with *alopecia areata*, suggesting a biologically plausible link between vitamin D pathways and disease activity [8]. Current management of localized *alopecia areata* relies mainly on corticosteroid-based approaches, which may induce regrowth but are limited by incomplete response, relapse after cessation, and concerns regarding adverse effects [9, 10]. Microneedling has gained attention as a simple office procedure that produces controlled micro-injury in the scalp, stimulates the release of growth factors, improves perifollicular vascularity, and increases penetration of topical preparations [11]. In parallel, topical vitamin D3 formulations have been proposed to support hair follicle cycling and to modulate local immune activity in *alopecia areata* [12]. Whether combining microneedling with topical vitamin D3 offers superior clinical benefit to microneedling alone in patients with patchy *alopecia areata* remains uncertain, and a controlled comparison is required to clarify the added value of topical vitamin D3 in this setting.

Although there are already others on the quality of life in parents, there is little literature on it that looks at parenting of children with intellectual disabilities within local contexts, especially taking into account such factors as work shifts, emotional stress, and support systems. This presents a research issue of how these factors affect their overall well-being and where they lack in support. This study aimed to evaluate the quality of life of parents, identify disparities depending on working shifts, and determine important factors influencing the physical, emotional, and social well-being to propose effective interventions.

METHODS

This study was a non-blinded randomized clinical trial comparing microneedling combined with topical vitamin D3 against microneedling alone in localized *alopecia areata*. The trial is registered at a clinical trial registry (NCT07243977). Participants and treating clinicians were not blinded; however, outcome assessment was performed by a dermatologist who was not involved in treatment delivery and remained blinded to treatment allocation. Recruitment and all procedures take place in the outpatient dermatology clinic of the Services Institute of Medical Sciences from April to October 2025, with a 1:1 allocation ratio between the two treatment groups. Approval has been obtained from the Institutional Review Board (IRB/2025/1571/SIMS; dated 29th March 2025), and written informed consent is secured from every participant before enrolment. Eligible participants are adults aged 18

to 60 years with clinically diagnosed localized *alopecia areata* involving less than 50 % of the scalp. The diagnosis is confirmed by dermoscopic features such as black dots, yellow dots, short broken hairs, tapering hairs, or vellus regrowth. Both sexes are included. Individuals must not have received topical or systemic treatment for *alopecia areata* during the 4 weeks preceding recruitment. Exclusion criteria comprise active bacterial, fungal, or viral scalp infection, visible scalp atrophy or scarring following previous intralesional steroid injections or other procedures, *alopecia totalis*, *alopecia universalis*, *ophiasis* pattern disease, or rapidly progressive *alopecia areata*. Patients with bleeding disorders or those on antiplatelet or anticoagulant therapy, as well as those with uncontrolled diabetes mellitus, severe hypertension, autoimmune diseases requiring systemic therapy, chronic liver disease, chronic kidney disease, or ongoing systemic immunosuppression, are also excluded. The sample size is calculated using the WHO calculator for comparison of two proportions, based on previously reported response rates of 4 percent for microneedling alone and 28 % for microneedling with topical vitamin D3, with a significance level of 5 % and power of 80 %, resulting in 80 participants, 40 in each group [13]. A computer-generated random sequence is used to assign participants to the two treatment arms in a 1:1 ratio, with allocation implemented in sequential order. Allocation concealment was ensured using sequentially numbered, opaque, sealed envelopes. Participants randomized to the microneedling plus vitamin D3 group (MN + VD3) receive treatment with an automated dermapen device (Dr. Pen, USA) fitted with adjustable needles set between 1.5 and 2.0 mm to reach the dermis overlying the follicular bulb. After cleansing the affected scalp areas with normal saline, microneedling is performed in vertical, horizontal, and diagonal directions until fine pinpoint bleeding appears, which is taken as the endpoint of each session. An aqueous cholecalciferol solution (Sunny D ampoule, 200,000 IU in 2 ml, equivalent to 2.5 mg/ml) is used as the topical vitamin D3 preparation. A small amount is applied to the lesion immediately before microneedling to facilitate transdermal delivery, and the remaining volume is spread evenly over the treated area once microneedling has been completed. The maximum dose per session is limited to 2.5 mg/ml [13]. Participants in the microneedling-alone (MN) group undergo the same procedure with the same device, needle depth, technique, and endpoint, but no active topical agent is applied before or after treatment. Standard cleaning and identical post-procedure care instructions are given to both groups, apart from the omission of vitamin D3 in the control arm. In each group, the treatment duration was 12 weeks (six sessions), and the primary endpoint assessment was performed at

week 12. Clinical response is assessed using the Severity of *Alopecia* Tool (SALT), which expresses the percentage of scalp surface affected by hair loss using categories S0 (no loss), S1 (1 to 24 %), S2 (25 to 49 %), S3 (50 to 74 %), S4 (75 to 99 %) and S5 (100 % loss). The area of involvement is estimated with the rule-of-thumb method, in which the patient's thumbprint approximates 1 % of the scalp, to provide consistent baseline and follow-up measurements. Percentage hair regrowth is calculated as: (SALT score at baseline minus SALT score at follow-up) multiplied by 100 and divided by the baseline SALT score. Responses (Efficacy) are further classified as poor (<25 % improvement), moderate (26 to 50 %), good (51 to 75 %), and excellent (≥ 75 % improvement) [14]. For the primary analysis, efficacy is defined as an excellent response at 12 weeks after initiation of treatment. Relapse is recorded when there is loss of newly regrown hair or the appearance of new *alopecia areata* patches during follow-up. Relapse assessment was confined to the treatment phase and the week 12 endpoint visit, as no extended post-treatment follow-up period was planned. Adverse events of interest include erythema, pain, bleeding, pigmentation changes, and pruritus. Participants attend six treatment visits at fortnightly intervals over three months; outcome evaluation is performed at the end of this period using a structured data collection form. Local reactions are managed according to a predefined protocol, and treatment is discontinued if any serious adverse event occurs that, in the investigator's judgment, makes further

sessions unsafe.

Data were entered and analyzed using SPSS version 23.0. Quantitative variables were summarized as mean and standard deviation. Qualitative variables were reported as counts and percentages. Between-group comparisons for categorical data were carried out with the chi-square test or Fisher's exact test. Independent samples t-tests were used to compare continuous measures between the two treatment arms. Changes in SALT scores over time within each group were examined using repeated-measures analysis of variance. Potential modifying factors such as age, sex, body mass index, and duration of symptoms were explored through stratification, followed by chi-square testing within strata. A p-value of less than 0.050 was regarded as statistically significant.

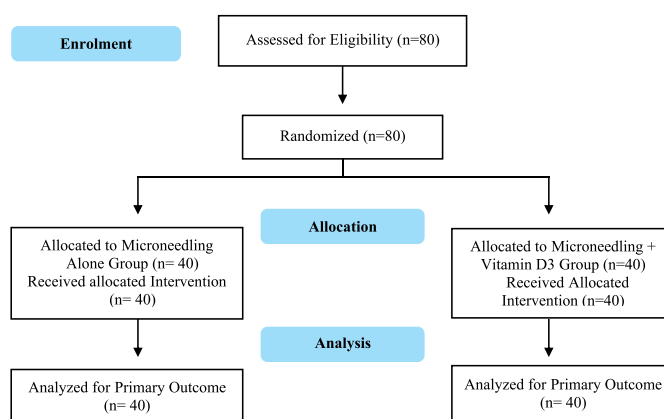


Figure 1: CONSORT Flow Chart

RESULTS

Baseline demographic variables and clinical characteristics were comparable between groups. The mean age was also similar between groups (35.08 ± 10.73 years in MN vs 36.78 ± 12.23 years in MN + VD3; $p=0.511$), showing that age distribution was balanced at baseline. Gender distribution was similar in both groups, with males constituting 62.5% in the MN group and 57.5% in the MN + VD3 group, while females constituted 37.5% and 42.5%, respectively ($p=0.648$). Mean disease duration was also comparable ($p=0.684$) (Table 1).

Table 1: Demographic Characteristics of the Study Population (n=80)

Demographic Variables	Total, n (%)	Microneedling Alone (MN), n=40	Microneedling + Vitamin D3 (MN + VD3), n=40	p-value
Age Group				
18–40 Years	51 (63.8%)	27 (67.5%)	24 (60.0%)	0.485
41–60 Years	29 (36.3%)	13 (32.5%)	16 (40.0%)	
Mean Age ± SD (Years)	35.93 ± 11.46	35.08 ± 10.73	36.78 ± 12.23	0.511
Sex				
Male	48 (60.0%)	25 (62.5%)	23 (57.5%)	0.648
Female	32 (40.0%)	15 (37.5%)	17 (42.5%)	
Disease Duration				
Up to 5 Months	50 (62.5%)	26 (65.0%)	24 (60.0%)	0.644
≥6 Months	30 (37.5%)	14 (35.0%)	16 (40.0%)	
Mean Duration ± SD (Years)	1.38 ± 0.49	5.13 ± 3.06	4.85 ± 2.97	0.684
Body Mass Index ± SD (kg/m²)	25.10 ± 3.48	25.04 ± 3.55	25.16 ± 3.45	0.886

Baseline SALT score was 19.85 ± 4.78 in the MN group and 20.20 ± 4.71 in MN + VD3, with statistically no significant difference

($p=0.743$), indicating similar disease severity at enrolment. The mean SALT score was significantly lower in the MN + VD3 group ($p=0.003$), demonstrating better results with combination treatment. Similarly, the mean absolute reduction in SALT score was also significantly greater in the MN + VD3 group compared with the MN group (13.27 ± 4.28 vs 9.66 ± 3.83 ; $p=0.001$), indicating that the addition of topical vitamin D3 resulted in a greater reduction in scalp hair loss (Table 2).

Table 2: SALT Score Changes and Hair Regrowth from Baseline to 12-Week Endpoint

Parameters	Microneedling Alone (MN) (n=40), Mean $\pm$ SD	Microneedling + Vitamin D3 (MN + Vd3), Mean $\pm$ SD	Mean Difference (95% CI)	p-value	Effect Size (Cohen's d)
Baseline SALT Score	19.85 $\pm$ 4.78	20.20 $\pm$ 4.71	0.35 (-1.76 to 2.46)	0.743	0.07
SALT Score at 12 Weeks	10.19 $\pm$ 5.18	6.93 $\pm$ 4.15	-3.26 (-5.35 to -1.17)	0.003	0.70
Absolute SALT Reduction	9.66 $\pm$ 3.83	13.27 $\pm$ 4.28	3.61 (1.55 to 5.67)	0.001	0.89
Percentage Hair Regrowth (%)	48.14 $\pm$ 24.05	65.74 $\pm$ 18.63	17.60 (8.02 to 27.17)	<0.001	0.82

At 12 weeks, the treatment response was significantly better in the MN + VD3 group than in the MN group ($\chi^2 = 10.265$, $p=0.016$). When responses were grouped, good or excellent hair regrowth was found in 50.0% of patients in the MN group compared with 82.5% in the MN + VD3 group ($\chi^2 = 9.534$, $p=0.002$), indicating that combination therapy reported better results compared to microneedling alone (Table 3).

Table 3: Treatment Response Classification at 12-Week Endpoint Based on Percentage Hair Regrowth (n=80)

Response Category	Microneedling Alone (MN) (n=40), n (%)	Microneedling + Vitamin D3 (MN + Vd3) (n=40), n (%)	χ^2	p-value	Effect Size
Poor (<25% regrowth)	7 (17.5%)	2 (5.0)	10.265	0.016	0.358
Moderate (26–50% regrowth)	13 (32.5%)	5 (12.5%)	—	—	—
Good (51–75% regrowth)	11 (27.5%)	14 (35.0%)	—	—	—
Excellent ($\geq 75\%$ regrowth)	9 (22.5%)	19 (47.5%)	—	—	—
Combined Good/Excellent ($\geq 51\%$ regrowth)	20 (50.0%)	33 (82.5%)	9.534	0.002	0.345

Overall comparison of the four response categories was performed using the Chi-square test (df = 3), and effect size is reported as Cramér's V. For the dichotomized comparison (Good/Excellent vs Poor/Moderate), the Chi-square test (df = 1) was applied, and effect size is reported as Phi (Φ).

Adverse events were mild and transient. Overall frequency of any adverse event was similar between groups (27.5% in MN group vs 32.5% in MN + VD3 group; $p=0.626$). No serious adverse events, systemic reactions, or treatment discontinuations occurred (Table 4).

Table 4: Adverse Events Frequency and Distribution by Treatment Group

Adverse Event	Microneedling Alone (MN) (n=40), n (%)	Microneedling + Vitamin D3 (MN + Vd3) (n=40), n (%)	χ^2	p-value
Erythema	9 (22.5%)	9 (22.5%)	0.000	<0.001
Pruritus	8 (20.0%)	8 (20.0%)	0.000	<0.001
Pinpoint Bleeding	5 (12.5%)	8 (20.0%)	0.827	0.363
Post-Inflammatory Hyperpigmentation	1 (2.5%)	3 (7.5%)	1.053	0.305
Any Adverse Event	11 (27.5%)	13 (32.5%)	0.238	0.626

Stratified comparisons for good/excellent response ($\geq 51\%$ regrowth) across age, sex, and disease duration subgroups are summarized (Table 5).

Table 5: Stratified Analysis of Excellent/Good Treatment Response ($\geq 51\%$ Hair Regrowth)

Stratification Variables	Sub-group	Microneedling Alone (MN), n (%)	Microneedling + Vitamin D3 (MN + Vd3), n (%)	χ^2	p-value
Age Group					
18–40 years	Yes	17 (63.0%)	22 (91.7%)	5.818	0.016*
	No	10 (37.0%)	2 (8.3%)		
41–60 years	Yes	3 (23.1%)	11 (68.8)	5.992	0.014*
	No	10 (76.9%)	5 (31.2%)		
Sex					
Male	Yes	12 (48.0%)	19 (82.6%)	6.273	0.012*
	No	13 (52.0%)	4 (17.4%)		
Female	Yes	8 (53.3%)	14 (82.4%)	3.124	0.077
	No	7 (46.7%)	3 (17.6%)		
Disease Duration					
Up to 5 Months	Yes	13 (50.0%)	19 (79.2%)	4.608	0.032*
	No	13 (50.0%)	5 (20.8%)		
≥6 Months	Yes	7 (50.0%)	14 (87.5%)	5.000	0.025*
	No	7 (50.0%)	2 (12.5%)		

An excellent/good response is defined as achieving $\geq 51\%$ hair regrowth from baseline to 12-week endpoint. Data presented as n

(%). Chi-square tests are employed for stratified comparisons. Asterisk (*) indicates $p < 0.050$. Fisher's exact test p -values are presented where appropriate due to small expected cell frequencies

DISCUSSION

This randomized trial showed that adding topical vitamin D₃ to microneedling improved outcomes in adults with localized *alopecia areata*. Baseline demographic features, disease duration, and SALT scores were similar in both groups, indicating successful randomization. Over 12 weeks, scalp involvement decreased with both regimens, but the reduction was greater with combination therapy: mean SALT at week 12 was significantly lower in the MN + VD3 arm than in the MN arm ($p = 0.003$), with a larger absolute fall from baseline. Expressed as percentage hair regrowth, microneedling alone achieved roughly 48% mean regrowth, whereas microneedling plus vitamin D₃ achieved about 66% ($p < 0.001$), corresponding to an absolute difference of approximately 18 percentage points. Categorical responses reflected the same pattern, with good or excellent regrowth ($\geq 51\%$ regrowth) observed in 82.5% of participants receiving MN + VD3 compared with 50.0% receiving microneedling alone ($p = 0.002$). Both regimens were well tolerated. Adverse effects were limited to mild, short-lived erythema or pruritus in about one-fifth of participants in each group, and no serious events, treatment discontinuations, or procedure-related complications occurred. Microneedling alone produced improvement in SALT scores in this trial, in keeping with prior reports that scalp micro-injury can be followed by regrowth in alopecia areata [14]. Mohammed described early regrowth within one month and near-complete recovery by three months in 20 patients treated with microneedling, without major toxicity [15]. Randomized comparisons with cryotherapy or intralesional agents have also reported SALT reductions over time [16, 17]. In the present study, microneedling alone was associated with improvement in SALT score and categorical response, although response rates were lower than those observed with adjunctive vitamin D₃. Ali et al. similarly reported higher-grade regrowth with MN + vitamin D₃ than MN alone [13]. Other vitamin D-based approaches also support a treatment role in *alopecia areata*. Intralesional cholecalciferol achieved at least 75% regrowth in about half of treated patients, with minimal response in saline controls [18], while topical calcipotriol produced high-grade regrowth in roughly two-thirds of treated sites and relapse rates comparable to or lower than clobetasol [19]. Small series and case reports describing near-complete regrowth and restoration of vitamin D receptor expression further strengthen the rationale for combining topical vitamin D₃ with microneedling [20, 21]. The present trial

supports the concept that vitamin D₃ acts as an effective adjunct when delivered through microneedling channels. Combination treatment produced a higher mean percentage regrowth and a greater proportion of good or excellent responders than microneedling alone, consistent with data from androgenetic *alopecia* in which microneedling enhances the effect of topical minoxidil and other agents [22]. Clinical evidence indicates that microneedling can augment drug delivery and improve responses to minoxidil, triamcinolone, platelet-rich plasma, and related therapies in hair-loss disorders [22–24]. Safety findings in this study, limited to brief erythema, pruritus, and pinpoint bleeding, are in line with previous microneedling and vitamin D-based literature, where local irritation and erythema are generally mild, and serious adverse effects, including atrophy or systemic complications, are uncommon [18, 19]. *Alopecia areata* is an immune mediated disorder in which collapse of follicular immune privilege permits T-lymphocyte infiltration around the hair bulb, leading to premature catagen and telogen transition. Vitamin D3 has been reported to influence antigen presentation and cytokine balance, with reduced Th1 signaling and increased regulatory mediators [25]. If enhanced delivery occurs through microneedling, local immune activity might be tempered, which could contribute to regrowth. Vitamin D receptor signaling also affects keratinocyte differentiation and hair cycling, and low vitamin D has been described in *alopecia areata* in patients [26]. Microneedling may assist hair regrowth by triggering a local repair response after controlled dermal injury. This process has been linked to the release of growth factors, stimulation of dermal papilla activity, activation of follicular stem cell niches, and improved perifollicular blood flow, which may support anagen entry and follicle persistence [11]. Experimental models also show upregulation of WNT pathway genes and even new follicle formation after repeated wounding [20, 27]. Creation of transient microchannels further enhances penetration of topical agents, providing a plausible explanation for the superior responses seen with microneedling plus vitamin D₃ in this trial [13]. However, comparison across studies is difficult because vitamin D has been delivered as intralesional cholecalciferol, topical calcipotriol, or microneedling-assisted solutions, while microneedling protocols differ in device, depth, and frequency, and outcomes range from simple regrowth scores to SALT-based measurements [22, 24]

Key strengths of this trial are its randomized design with well-balanced baseline characteristics, use of validated SALT scoring with both continuous and categorical outcomes, stratified analyses by age, sex, and disease duration, and prospective recording of adverse events that

support a reliable safety profile. This trial has important constraints, including a sample of 80 patients from a single centre, a lack of blinding, and follow-up limited to twelve weeks. Serum vitamin D levels were not measured, which limited the correlation of treatment response with vitamin D status. Stratified comparisons and multiple analyses were performed, which may increase the risk of type I error due to multiple comparisons. Despite these limitations, microneedling with topical vitamin D3 produced greater SALT improvement, higher mean regrowth, and better or excellent responses than microneedling alone without additional clinically relevant adverse events. Larger multicenter studies with longer follow-up and quality of life outcomes are required to confirm these findings.

CONCLUSIONS

This study suggests that adding topical vitamin D₃ to microneedling may improve treatment response in localized patchy *alopecia areata* compared with microneedling alone. A higher proportion of patients achieved clinically meaningful hair regrowth with combination therapy, and the benefit was observed across the main subgroups assessed by age, sex, and disease duration. These findings support topical vitamin D₃ as a potential adjunct to microneedling in localized disease; however, confirmation in larger blinded trials with longer follow-up is required.

Authors' Contribution

Conceptualization: RM

Methodology: FA, HT, UA, AUH

Formal analysis: RM, HT, UA

Writing and Drafting: RM, FA, HT, UA, AUH

Review and Editing: RM, FA, HT, UA, AUH

All authors approved the final manuscript and take responsibility for the integrity of the work

Conflicts of Interest

All the authors declare no conflict of interest.

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