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Report

Correlation of vitamin D and vitamin D receptor expression in patients with alopecia areata: a clinical paradigm

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Abstract

Background Vitamin D (Vit.D) deficiency has been reported in alopecia areata (AA). Downregulation of Vitamin D receptor (VDR) on hair follicles is associated with reduced hair growth.

Objective To correlate serum Vit.D levels with severity, pattern, and duration of AA, and density of VDR expression over hair follicles in AA patients.

Methods Prospective study including 30 AA patients and 30 healthy controls. Clinical details and serum Vit.D measurement and scalp biopsy for histopathology and VDR expression was performed in patients and controls at baseline and after 6 months of treatment of AA.

Results Mean age of patients and controls was 28.9 ± 9.96 and 31.17 ± 9.43 years, respectively. Mean SALT score in patients was 35.8 ± 27.5 with a median disease duration of 48 weeks. Mean serum Vit.D levels was 7.65 ± 4.50 ng/ml and 15.8 ± 11.47 ng/ml in patients and controls, respectively. Twenty-nine (96.7%) patients were Vit.D deficient (<20 ng/ml), compared to 22 (73.3%) controls ($P = 0.001$). Serum Vit.D levels inversely correlated with severity of the disease ($r = -256$), $P = 0.17$, and duration of disease but did not correlate with pattern of AA and VDR expression in tissue samples. VDR expression was reduced in all patients and was normal in controls. Inverse correlation of VDR was noted with presence of inflammation on histology ($P = 0.02$). VDR upregulation post treatment was seen only in 13% of patients and demonstrated no correlation with response to treatment.

Conclusion Vit.D deficiency in AA correlates inversely with disease severity and duration. VDR expression is reduced in AA and inversely correlate with inflammation histologically but does not correlates with serum Vit.D levels, severity, pattern, or duration of illness.

Introduction

Alopecia areata (AA) is an autoimmune inflammatory disorder of hair of unclear etiology. Vitamin D (Vit.D) plays an important role in calcium homeostasis, immune regulation, and cell growth and differentiation.[1] The active form of Vit.D mediates its action by binding to specific Vit.D receptors (VDR) located in the nuclei of target cells. Studies have proposed a connection between few autoimmune diseases and Vit.D deficiency, suggesting Vit.D deficiency might be an environmental stimulus for induction of autoimmunity. It has been demonstrated that VDR is strongly expressed on human and murine hair follicles,[2] and VDR expression in keratinocytes is necessary to maintain a normal hair cycle.[2] A lack of VDR is associated with reduced epidermal differentiation and hair follicle growth.[2] In addition, patients with hereditary 1,25-dihydroxy Vit.D3-resistant rickets type II and VDR knockout mice exhibit a phenotype that includes alopecia. We aimed to assess serum levels of Vit.D in AA patients and observed if there was any correlation of serum level of Vit.D with severity and duration of AA and also on VDR expression over hair follicles.

Materials and methods

This was a prospective study wherein 30 patients with AA and 30 age- and sex-matched healthy controls were included. Exclusion criteria was patients with associated skin diseases like atopic dermatitis, psoriasis, autoimmune diseases (lupus erythematosus, rheumatoid arthritis, scleroderma, and thyroid disorder), patients taking oral corticosteroids in previous 2 weeks, patients applying topical Vit.D analogs or patients who have received regular Vit.D supplementation in previous 6 months, and pregnant and lactating mothers.

After ethical clearance and informed consent, detailed history regarding duration and extent of disease, family history, and past treatment taken was recorded on prefixed proforma. Complete clinical examination including SALT scoring[3] was done. Subcategories of SALT score were defined as S0 = no hair loss, S1 = <25% hair loss, S2 = 25–49% hair loss, S3 = 50–74% hair loss, S4 = 75–99% hair loss, and S5 = 100% hair loss. Serum Vit.D level of patients and controls was measured, which was further categorized as: sufficient (>30 ng/mL), insufficient (20–30 ng/ml), and deficient (<20 ng/ml).[4] Histopathological examination and immunohistochemical (IHC) staining of tissue specimen was performed to observe VDR expression over hair follicle and epidermis and was compared with controls. Immunoreactivity was evaluated in a semiquantitative manner by analyzing both staining intensity and percentage of positive cells. At least five hair follicles were studied per slide. Slides were assessed by a blinded dermatopathologist (UN) for percentage of cells stained and were scored as: No positivity – zero, 10–25% positive – 1+, 26–50% positive – 2+, 51–75% positive – 3+, and >75% positive – 4+. Patients found to be Vit.D deficient were given oral Vit.D supplementation in the form of oral cholecalciferol 60,000 IU once weekly for 12 weeks. All patients were treated as per standard guidelines[5] and followed up regularly at monthly intervals for 6 months. Change in SALT score was calculated and categorized as <25%($SALT_{<25}$), 26–50% ($SALT_{26\text{-}50}$), and >50%($SALT_{>50}$). At the end of 6 months, serum Vit.D levels were again measured and a scalp punch biopsy repeated from the same patch where pretreatment biopsy was done to see for change in VDR expression at area where new growth has occurred.

Results

The clinical–epidemiological profile of patients and controls is summarized in Table 1. SALT score in patients ranged from 10 to 100 with a mean SALT score of 35.8 ± 27.5, S1-S2, S3-S4, and S5 disease was noted in 24 (80%), 3 (10%), and 3 (10%) patients, respectively (Table 2).

Vit.D and AA

Mean serum Vit.D level in patients and controls was 7.65 ± 4.50 ng/ml and 15.8 ± 11.47 ng/ml, respectively. Twenty-nine (96.7%) patients and 22 (73.3%) controls were found to be Vit.D deficient, which was a statistically significant difference, $P = 0.001$ (Fig. 1). Serum Vit.D levels inversely correlated with severity of AA. SALT scores were higher in patients with lower serum Vit.D ($r = -0.256$) (Fig. 2), $P = 0.17$. Serum Vit.D levels also inversely correlated with total duration of illness ($P = 0.03$), $r = -.224$ (Fig. 3). No correlation could be demonstrated between serum Vit.D levels and site, pattern of disease, and progressive or stable disease.

After completion of a 12-week of treatment period, complete hair regrowth was seen in 8 (27%) patients at 6 months. Post supplementation, mean Vit.D level of patients was 21.97 ng/ml. We did not find any significant correlation between change in SALT scores and serum Vit.D levels post supplementation.

Table 1 Clinico-epidemiological details of patients and controls

	Patients	Controls
Females	19	14
Males	11	16
Mean age (years)	28.97 ± 9.96	31.17±9.43
Median duration of disease (weeks)	48	–
Patchy AA	22 (73.3%)	–
Other patterns AA (diffuse, alopecia totalis, ophiasis, alopecia universalis)	8 (26.7%)	–
Involvement of other body sites	8 (26.7%)	–
First episode of AA	12 (40%)	–
More than one episode of AA	18 (60%)	–
Progressive disease (appearance of new patches and increase in size of existing patches)	22 (73.3%)	–
Mean SALT score	35.8 ± 27.5	–
Positive hair pull test	21 (70%)	–
Exclamation mark hair	22 (73%)	–
Nail involvement	13 (43%)	–

Table 2 SALT subclasses of AA patients

SALT subclass	No. of patients (*N*)	Males	Females
S1–S2	24 (80%)	11	13
S3–S4	3 (10%)	0	3
S5	3 (10%)	0	3

Histopathology and VDR expression in AA

Histopathologic examination and VDR expression on IHC was studied in 29 pretreatment biopsy samples and 15 posttreatment biopsy samples. The inflammatory infiltrate was absent in 8 (27.5%), mild in 15 (51.7%), moderate in 6 (20.6%), and severe in none. Perifollicular fibrosis was mild in 14 (48.2%), moderate in 10 (34.4%), severe in 4 (13.7%), and absent in 1 (3%).

Pretreatment VDR expression was found in 48.2% cases in hair follicles, but the intensity of positivity was less as compared to controls (Table 3). In hair follicles, the intensity of IHC was 1+ in 8 (27.5%) patients, 2+ in 5 (17.2%) patients, and 3+ in 1 (3%) patient. Pretreatment VDR positivity did not differ with respect to duration of illness. VDR positivity was found in 12 (57.1%) patients with patchy AA, while only 3 (37.5%) patients with other patterns of AA (ophiasis, diffuse, alopecia universalis); however, it was not statistically significant (Table 4). Seven (63%) patients with first episode AA were VDR positive, while 8 (44%) patients with more than one

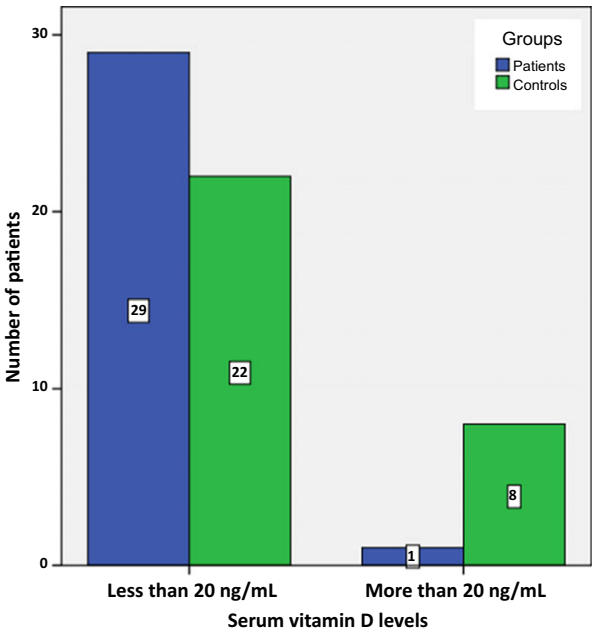

Figure 1 Comparison of Vitamin D levels of patients and controls

episode also had VDR positivity in biopsy specimens. Among the study cohort, it was observed that the only patient who had sufficient serum Vit.D level demonstrated absence of VDR expression on IHC, which was contrasting to those patients who were serum Vit.D deficient but still demonstrated VDR on IHC. Thus, VDR expression might not be related completely to serum Vit.D levels only.

On correlating with histopathological examination, VDR positivity was typically noted in those patients with absent or mild inflammation ($P = 0.02$). Among patients having absent, mild, or moderate inflammatory infiltrate in scalp skin, 9 (56%), 6 (75%), and 0% patients, respectively, had VDR expression in lesional scalp ($P = 0.02$). There was a significant correlation between perifollicular fibrosis and VDR as zero, 6 (42.9%), 9 (90%), and 0% patients with absent, mild, moderate, and severe fibrosis, respectively, had VDR expression positivity ($P = 0.009$), $r = +0.212$.

When disease parameters and VDR expression were compared in 15 posttreatment biopsy samples with their baseline counterpart, VDR expression was lost in 11/15 samples. Upregulation of VDR expression was observed in only two patients; in the first patient, VDR expression was absent at baseline but upregulated to 25–50% (2+) of hair follicle keratinocytes at the end of 6 months (Fig. 4). At the end of 6 months, the patient had a complete clinical cure of AA and histopathologically no inflammation or fibrosis, and normal anagen/telogen ratio. In the second patient, there was increase in VDR positivity at the end of the study (2+) compared to baseline (1+). This patient had clinical cure at the end of the study with histopathologically no inflammation**.** Seven patients who had weak positivity of VDR at baseline demonstrated a complete absence of VDR expression at the end of the study despite clinical improvement with a decrease in SALT score by 25–30%. All these patients demonstrated the presence of a mild to moderate degree of lymphocytic perifollicular inflammation on histopathology at the end of the study. In 4/15 patients, there was VDR absence both at baseline and the end of the study despite clinical

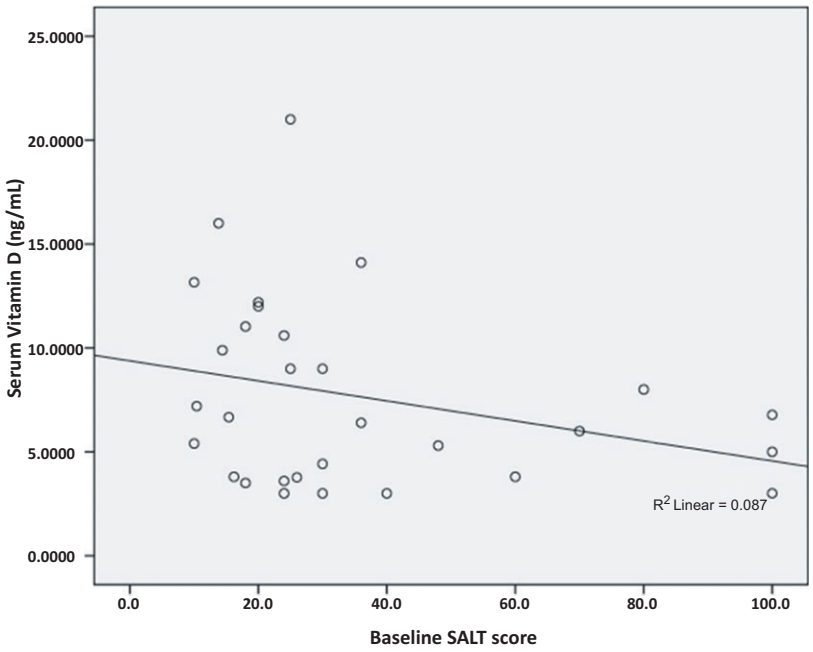

Figure 2 Correlation of serum Vitamin D levels with SALT score

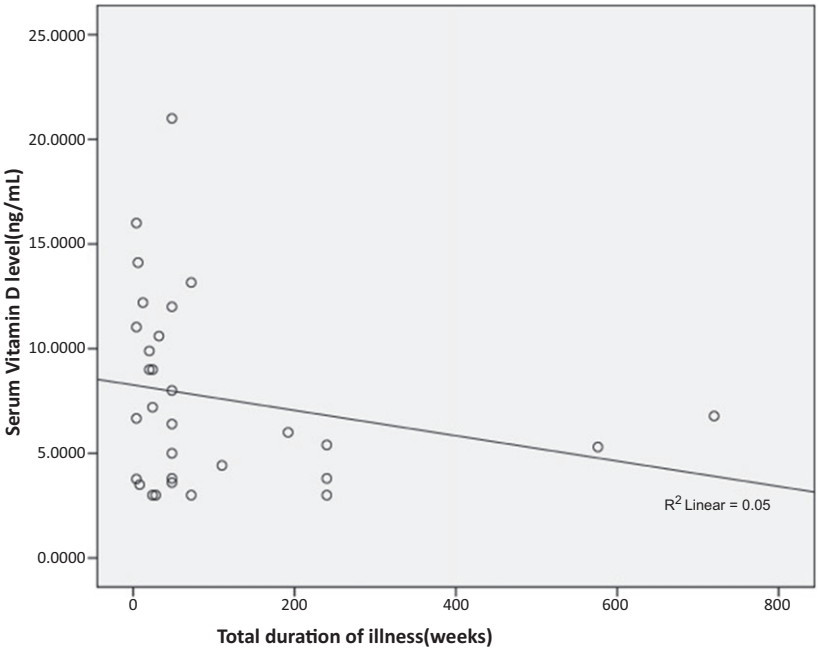

Figure 3 Correlation of serum Vitamin D with total duration of illness

Table 3 VDR expression positivity in hair follicles of pre- and posttreatment

Variables	Controls	Pretreatment	Posttreatment (6 months)
No. of biopsy specimens	5	29	15
VDR expression positivity in hair follicles	100%	14/29 (48.2%)	4/15 (26.6%)
VDR expression positivity in epidermis	100%	9/29 (31.3%)	4/15 (26.6%)
VDR expression positivity in sebaceous glands	100%	2/29 (6%)	2/15 (13.3%)
VDR expression positivity in eccrine glands	100%	16/29 (55%)	10/15 (66%)

Table 4 Comparison of VDR positivity with different disease characteristics, disease severity, and serum vitamin D levels

Variables	VDR positive	VDR negative	*P* value
Males	5 (50%)	5 (50%)	0.89
Females	10 (52.6%)	9 (47.4%)	
Duration of illness <1 year	11 (55.0%)	9 (45.0%)	0.59
Duration of illness >1 year	4 (55.6%)	4 (44.4%)	
Patchy AA	12 (57.1%)	9 (42.9%)	0.34
Other patterns of AA	3 (37.5%)	5 (62.5%)	
First episode of AA	7 (63.6%)	4 (36.4%)	0.31
More than one episode of AA	8 (44.4%)	10 (55.6%)	
Scalp only disease	9 (52.9%)	8 (47.1%)	0.87
Other sites also involved	6 (50%)	6 (50%)	
Progressive disease	10 (47.6%)	11 (52.4%)	0.47
Nonprogressive disease	5 (62.5%)	3 (37.5%)	
Vitamin D sufficient group	0 (0%)	1 (100%)	0.29
Vitamin D deficient group	15 (53.6%)	13 (46.4%)	
SALT subclass S1-S2	13 (56.5%)	10 (43.5%)	0.15
SALT subclass S3-S4	0 (0%)	3 (100%)	
SALT subclass – S5	2 (66.7%)	1 (33.3%)	
Inflammation			
Mild	9 (56.3%)	7 (43.8%)	0.02
Moderate	0 (0%)	5 (100%)	
None	6 (75.0%)	2 (25.2%)	
Fibrosis present			
None	0 (0%)	1 (100%)	0.009
Mild	6 (42.9%)	8 (57.1%)	
Moderate	9 (90%)	1 (10%)	
Severe	0 (0%)	4 (100%)	

improvement, but they had mild inflammation still persistent on histopathology. In 2/15 patients, VDR expression was the same pre and post treatment despite clinical improvement and absent inflammation.

Discussion

AA is a common disease encountered by dermatologists, with a frequency ranging from 0.7 to 3.8% of patients attending dermatology clinics.[6] Vit.D reduces the function and differentiation of T helper 17 cells which are immune regulatory cells. VDR is expressed in the outer root sheath (ORS), hair follicle bulb, and the sebaceous gland in the hair follicle and participate in differentiation of hair follicles in utero.[7,8]

Sakai *et al.*[9] demonstrated that alopecia in VDR-null mice is because of inability of VDR-null keratinocytes to proliferate and failure of dermal papilla cells activation in response to anagen initiation; they proposed this defect may be because of absence of ligand-independent effects of VDR in vivo.[9] Xie *et al.*[2] demonstrated that both interfollicular epidermis and hair follicle

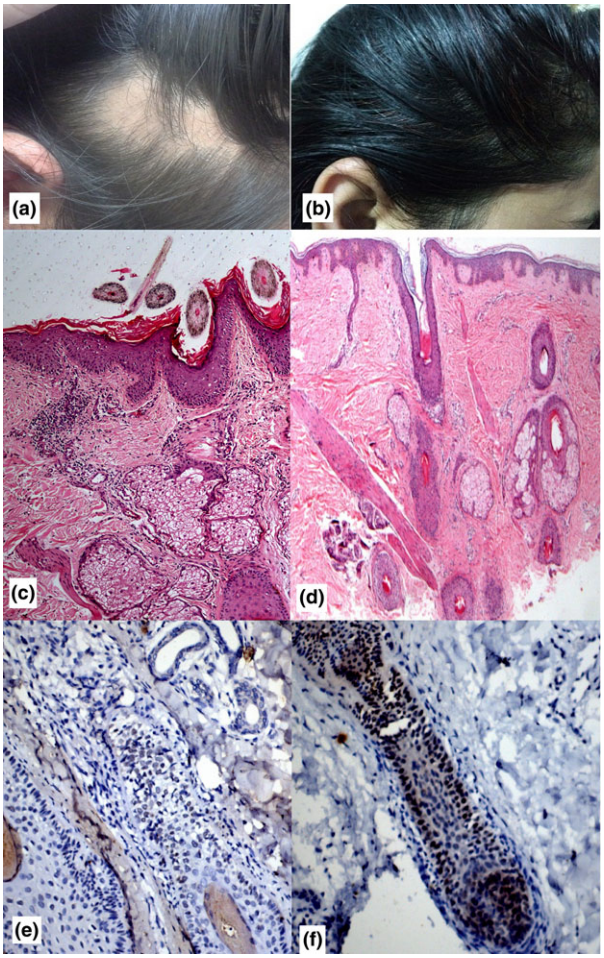

Figure 4 (a) Clinical photograph at baseline. (b) Clinical photograph at 6 months post treatment. (c) Moderate inflammatory infiltrate at baseline with decreased hair follicles and atrophic sebaceous glands. (H&E stain ×20). (d) Absence of inflammation and normal hair follicle at 6 months post treatment (H&E stain ×10). (e) Absence of VDR expression in hair follicle at baseline (Immunohistochemistry (IHC) for vitamin D3 receptor (VDR) – ×40). (f) Presence of VDR expression nuclear positivity in outer root sheath of hair follicle at 6 months post treatment (Immunohistochemistry (IHC) for vitamin D3 receptor (VDR) – ×40)

require Vit.D receptor for normal differentiation. Akar *et al.*[10] suggested that there is no relationship between VDR gene polymorphisms and AA.

Studies performed initially by Aksu Cerman et al.,[11] Mahamid et al.,[12] Yilmaz et al.,[13] and Robert D'Ovidio et al.[14] uniformly demonstrated reduced serum Vit.D levels in AA compared to healthy controls.

Similarly we found that serum Vit.D level of AA patients was significantly lower than controls, showing that Vit.D deficiency could be related to the disease pathogenesis or disease course. One hypothesis may be that people deficient in Vit.D are more prone to develop autoimmunity thus secondarily predisposed to develop AA and are psychologically distressed, and avoid exposure in public and in open air, which may further lead to decreased sun exposure and reduced Vit.D synthesis.

We found inverse correlation of Vit.D with duration of AA and a positive correlation with severity. From this it can be hypothesized that Vit.D has a role in pathogenesis of AA, as patients with Vit.D deficiency run a longer course of disease and it takes longer for autoimmunity to settle down despite multiple immunosuppressive therapies.

We observed no added benefit of Vit.D supplementation on clinical improvement and VDR upregulation. The reason for this might be that serum values more than 50 ng/ml are often required for its immunomodulatory actions,[15] which probably could not be achieved in our patients. Another explanation could be that the VDR might take more time for upregulation once they have been depleted after serum levels are replenished.

Uniquely we observed a positive correlation between VDR expression and absence of inflammation, a finding not highlighted in literature to the best of our knowledge. VDR positivity correlated inversely with severity of inflammation seen on histology. Thus, we hypothesize that the presence of inflammation could be a factor that prevents expression of VDR on keratinocytes, and absence of VDR may be an indicator for either presence of disease activity or relapse if the disease is not completely cured, thus this feature could be used as a monitoring tool. To confirm this, IHC investigation needs to be repeated after another 6 months to see the status or whether the still absence of VDR expression could be a silent representation of disease being active histologically needs to be proved by further studies. Another reason might be that VDR in epidermal and hair keratinocytes function independent from ligand interaction, which has been shown in previous studies in mice. We observed that there was loss of VDR expression as compared to baseline in patients with improving SALT score, and the reason for this might be continuing inflammation and autoimmunity. DH Kim et al.[16] demonstrated reappearance of VDR expression after treatment with topical Vit.D analog in a patient after 3 months; however, they did not perform histopathological examination in their patient. The authors hypothesized that topical calcipotriol may cause upregulation of receptors. Lim et al.[17] have also shown that VDR expression is decreased in hair follicle and epidermal keratinocytes leading to suppression of Wnt/beta catenin signals and cell differentiation. Decreased expression of VDR is related to suppression of the Wnt signaling pathway in AA.[18] This reduction in VDR expression and downregulation of the Wnt/b-catenin signals may evoke AA.[19]

Fawzi et al[20] have shown that there is decreased expression of tissue and serum VDR by ELISA in patients of AA, and androgenetic alopecia as compared to controls; they also found a negative correlation of tissue VDR and extent of AA. In our study, also, we did not find any correlation between extent of

AA and VDR expression in lesional tissue. Difference in result might be attributed to sensitivity of different method used for studying the VDR expression. They did not perform histopathological examination in their patients to look for the presence of inflammation.

Conclusion

Our study exemplifies that Vit.D does have a role in the pathogenesis of AA, and the VDR expression does not necessarily correlate with the clinical severity and serum Vit.D levels. Persistence of inflammation and absent VDR expression despite clinical remission need to be further studied. These could also be markers of relapse, which will need more studies.

Acknowledgments

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