Original article:

Evaluation of Status of Serum 25 Hydroxy Vitamin Dwith Alopecia Areata: A Case- Control Investigation

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Abstract

Introduction: Alopecia Areata is characterized by T-cell infiltrates and cytokine production around anagen-stage hair follicles. It is organ-specific autoimmune disease that causes inflammation around anagen-stage hair follicles. The aim of the study is to evaluate the status of serum vitamin D with Alopecia Areata.

Materials and Methods:It is a case- control investigation conducted on total 100 patients in which fifty patients suffering from Alopecia Areata and 50 were healthy control subjects. The separated serum was used for determination of 25(OH)D. Serum 25(OH)D concentration, the major circulating form of vitamin D, was measured using commercial Enzyme Linked Immunosorbent Assay (ELISA) kits. The data were analyzed using SPSS version 21. A P value< 0.05 was considered as statistically significant.

Results:There was no significant relationship between the 25(OH)D level and gender of patients, disease duration, disease recurrence, duration of sun exposure/day, or family history of AA. There was no significant association in serum 25(OH)D levels between patients with previous history of systemic corticosteroid therapy and patients who did not receive systemic corticosteroid treatment.

Conclusion:Serum 25(OH)D levels were negatively correlated with disease severity and pattern of hair loss.

Keywords: AlopeciaAreata, Cytokine, Anagen-Stage, Serum 25(OH)D, Corticosteroid.

INTRODUCTION:

Alopecia areata (AA) is a common organ-specific autoimmune disease that causes inflammation around anagen-stage hair follicles and leading to chronic and relapsinghair loss. it is associated with increased risk of other autoimmune disorders. AA is characterized by T-cell infiltrates and cytokine production around anagen-stage hair follicles.[1,2]The onset may be at any age and there is no known race or gender preponderance. It usually presents as patches of hair loss on the scalp but any hairbearing skin may also be involved.[3]Vitamin D is steroid hormone that plays an important role in calcium homeostasis and bone health. It has three sources: endogenous synthesis in the skin, which is induced by UVB radiation, dietary intake, and vitamin D supplementation.

Vitamin D is synthesized in the epidermal keratinocytes under effect of UV-B lights (290–315 nm) or ingested in diet and dietary supplements.[4] Vitamin D was found to have immune-regulatory effects. 1,25-Dihydroxy vitamin D3 (1,25(OH)2 D3) which is the active form of vitamin D, is one of the regulators of both innate and adaptive immune responses as it modulates immune functions and

activities of both T-lymphocytes and B-lymphocytes.[5] 1,25(OH)2 D3 has an important role in hair follicle biology. Vitamin D receptors (VDR) expression in epidermal keratinocytes and the mesenchymal dermal papilla cells were detected,[6]Although many different causes were suggested, the exact underlying etiology of AA is problematic. However, immunological, environmental, psychological, and genetic factors are the most powerful explanations.[7]

Studies from the last decade have clearly demonstrated that AA is an autoimmune disease mediated by T lymphocytes in which autoantigens are necessary to activate T cells that generate the disease.[8] Animal experimental models of AA have shown complex immunological changes, such as inflammatory infiltrate consisting of CD4>CD8 lymphocytes, macrophages and B cells, and a local increase in the cytokines interleukins-2 and 6.[9,10] While the treatment for patchy AA is effective, treatmentfor widespread disease is unsatisfactory. The available options are topical steroids, topical minoxidil, intralesional steroids and, for extensive disease, oral or pulse steroid therapy.[11,12]

Although the effect of vitamin D on keratinocytes and its effect on the pathogenesis of different dermatologic disorders is studied and reviewed.[6,13]

On the other hand, it has been demonstrated that vitamin D receptors (VDRs) are strongly expressed in the key structures of hair follicles. Expression of VDRs on keratinocytes is necessary for maintenance of the normal hair cycle.[14]It has also been shown that a lack of VDRs reduces epidermal differentiation and hair follicle growth.[15] Several studies were done to evaluate the role of vitamin D in different hair disorders with contrasting results.[16-18]

There is not enough data regarding vitamin D effects on AA. The aim of this study was to find the association between AA and level of vitamin D.

Materials and Methods

This was a case control study conducted on total 100 patients in which fifty patients suffering from Alopecia Areata and 50 were healthy control subjects. This study was carried out at Department of Dermatology, Era's Lucknow Medical College & Hospital, LucknowUP. Every selected subjects had skin phototype III and IV, included in case and control study.Disease duration was calculated from onset to the time of sampling. For cases with recurrent disease, the duration was calculated from the onset of relapse to the time of sampling. Clinical data describing patients' demographics (age and gender) as well as the clinical variables (site, disease recurrence, disease duration, positive family history, and duration of sun exposure/day) were all documented. If the diagnosis was not clear after clinical evaluation, a skin biopsy was performed to confirm the diagnosis.

A written consent was obtained from every individual of case and control study after the procedure had been fully explained. The study protocol was approved by the Ethical Research Committee of the College.

Exclusion criteria

- Patients taking vitamin D or calcium supplements,
- Patients which are smokers and taking Frequently alcohol,
- Patients with gastrointestinal problems, bone or renal disease, sarcoidosis, or any other
- Metabolic and systemic disorder that could affect 25(OH)D3 absorption, metabolism, or serum level,

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- Treatment by systemic or topical steroids, barbiturates, bisphosphonates, sulfasalazine,
- Patients with a history of diabetes mellitus, anemia, thyroid disorders, chronic liver or renal diseases, atopy, parathyroid disorders, patients with known autoimmune diseases or cancer,
- Patients with congenital or acquired errors of calcium or phosphorus metabolism, subjects with inadequate sun exposure and obese subjects were excluded.
- Pregnant or lactating women, subjects with skin phototypes V, and VI, and were also excluded from the study.

Sample taking procedure were explained as, Three milliliters of venous blood were withdrawn fromantecubital vein of each participant. Samples were thentransferred into a plain tube, left to stand for 30 min, and then centrifuged. All reagents and samples were usedat room temperature (18–26°C) and mixed gently to avoid foam formation. The separated serum was used for determinationof 25(OH)D. Serum 25(OH)D concentration, the major circulating form of vitamin D, was measured using commercial EnzymeLinked Immunosorbent Assay (ELISA) kits (ImmunodiagnosticSystems Limited, Bolden, UK).

The data were analyzed using SPSS version 21. The chi-square test was used to test differences in categorical variables between the two groups. A P value< 0.05 was considered as statistically significant.

RESULTS

A total 100 patients were included in this study in which 50 patients with Alopecia Areata and 50 who were in control group. In AA group 58% were male and 42% were females, and in healthy control group 54% were male and 46% were female. The Mean age is 22.37± 11.2 in AA group and 24.73 ± 9.6 in control group. The Mean of Body Mass Inder (Kg/m^2) is 21.16 ± 3.9 in AA group and 23.4 ± 5.61 in control group. 36% patients in AA group had III skin phototype and 64% had IV skin phototype, and in control Group 42% had III skin phototype and IV skinphototye had 58% subjects. this socio-demographic and clinical data of the studied groups were shown in Table 1 and Figure 1-5.

The mean of duratrion of disease is 3.46 months. The scalp was affected by alopecia areata in 76% cases Total body hair affected in 14% cases and 10% were beard. The pattern of hair loss found patchy in 56% cases, Totalis in 28% cases and ophiasis in 16% Cases. Mild AA found in 40% cases, moderate in 34% cases and Sever in 26% cases. The positive family history found in 22% cases and 39% found negative family history. These disease criteria in the studied cases are summarized in Table 2.

There was no significant relationship between the 25(OH)D level and gender of patients, disease duration, disease recurrence, duration of sun exposure/day, or family history of AA.

There was no significant association in serum 25(OH)D levels between patients with previous history of systemic corticosteroid therapy and patients who did not receive systemic corticosteroid treatment.(Table 3).

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Table 1: Demographic and laboratory data of AA and control group			
Variable	AA Group (50)	Control g (50)	P value
Gender n (%)			
Male	29 (58%)	27 (54%)	>0.05
Female	21 (42%)	23 (46%)	
Age (Years)			
Mean ± SD	22.37 ± 11.2	24.73 ± 9.6	>0.05
Body Mass Inder (Kg/m^2)			
Mean ± SD	21.16 ± 3.9	23.4 ± 5.61	>0.05
Skin Photo type			
III	18 (36%)	21 (42%)	>0.05
IV	32 (64%)	29 (58%)	
Sun Exposure/day (n)			
<2	16 (32%)	14 (28%)	>0.05
>2	34 (68%)	36 (72%)	

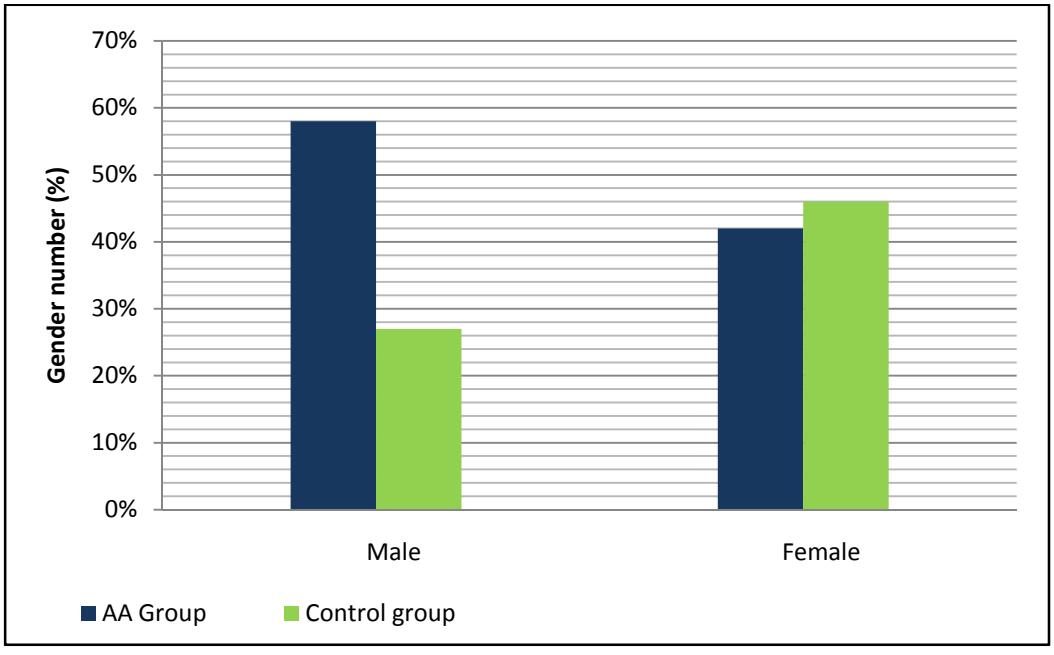

Figure 1: Gender distribution between two groups.

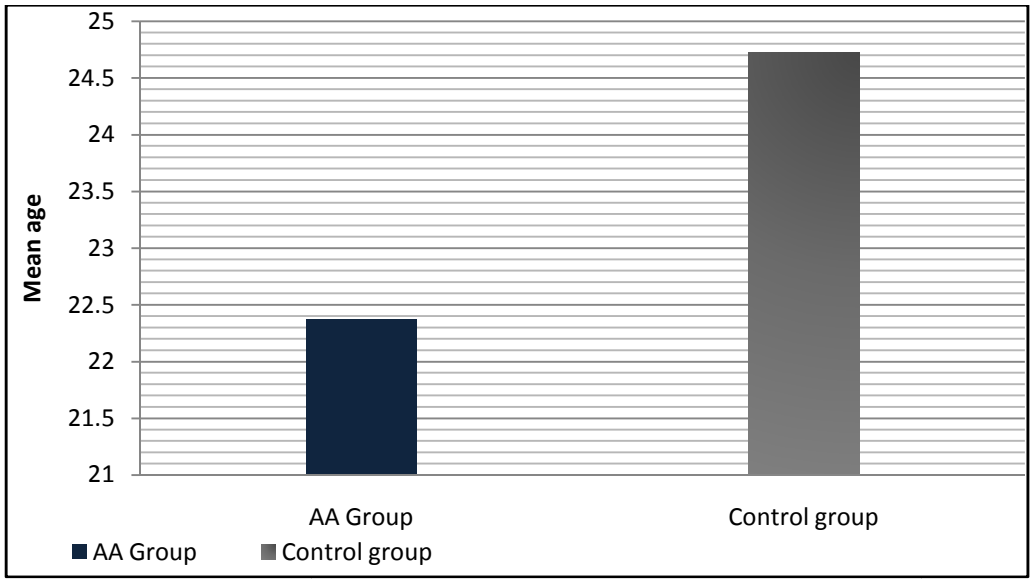

Figure 2: Comparison of age between two groups

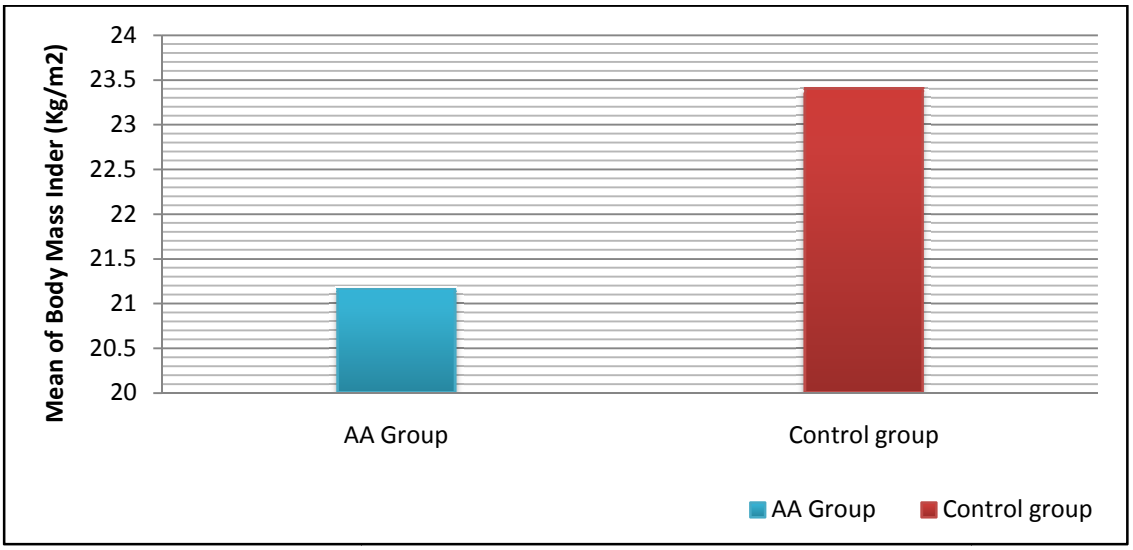

Figure 3: Comparison of BMI between two groups

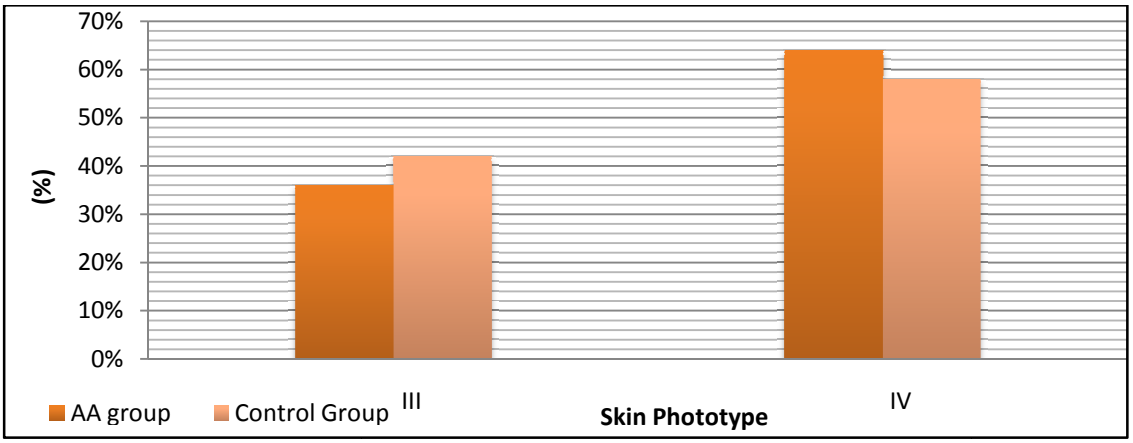

Figure 4: Comparison of skin photo type between two groups

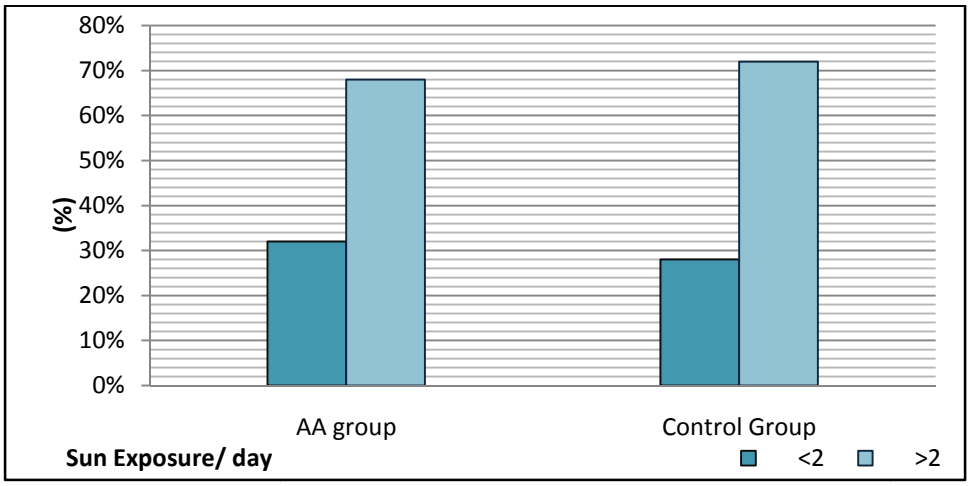

Figure 5:Comparison between the duration of sun exposure between two groups

Table 2: Disease criteria of the studied cases	
Variable	Mean±SD
Duration of disease (months)	
Mean±SD	3.46 ± 1.98
Range	
Site affected by alopecia areata, n (%)	
Scalp	38 (76%)
Beard	5 (10%)
Total body hair	7 (14%)
Pattern of hair loss, n (%)	

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Patchy	28 (56%)
Ophiasis	8 (16%)
Totalis/universalis	14 (28%)
Severity of the disease, n (%)	
Mild	20 (40%)
Moderate	17 (34%)
Severe	13 (26%)
Family history, n (%)	
Positive	11 (22%)
Negative	39 (78%)
Recurrence, n (%)	
Recurrent	13 (26%)
Nonrecurrent	37 (74%)
Past history of systemic corticosteroid, n (%)	
Corticosteroid treated	19 (38%)
Corticosteroid nontreated	31 (62%)

Table 3: Relationship between Vitamin D levels among the studied cases with regard to previouscorticosteroid treatment

Vitamin D level (nmol/L)	Studied group		P Value
	Corticosteroidtreated (n=19)	Corticosteroidnontreated (n=31)	0.21
Deficient/insufficient	16 (84.21%)	25 (80.64%)	
Normal	3 (15.78%)	6 (19.35%)	

DISCUSSION

In the present study, there was no significant relationship between the 25(OH)D level and gender of patients, disease duration, disease recurrence, duration of sun exposure/day, or family history of AA.There was no significant association in serum 25(OH)D levels between patients with previous history of systemic corticosteroid therapy and patients who did not receive systemic corticosteroid treatment.

Vitamin D is of particular interest to dermatologists for two important reasons: it is synthesized in the

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skin upon exposure to UV light, and it is an important treatment option for psoriasis and other skin diseases.[19]

Studies have shown that vitamin D has dose-dependent effects in the skin, including the modulation of growth factor and cytokine synthesis and signaling.[20]It is well known that VDR expression in keratinocytes is necessary for maintenance of the normal hair cycle, and a lack of VDRs reduces epidermal differentiation and hair follicle growth. In light of this information, Kim et al.[20] described a 7-year-old male with AA and reduced VDR expression in which recovery was observed following the topical application of calcipotriol, a potent vitamin D analog. In addition, decreased serum vitamin D levels in patients with AA were inversely correlated with disease severity, which may indicate a causal role for vitamin D deficiency in the pathogenesis of the disease. They suggested that vitamin D supplementation could be a reasonable and specific treatment strategy for AA.

The active form of vitamin D, 1,25-dihydroxyvitamin D3, mediates its action by binding to specific vitamin D receptors located in the nucleus of target cells. VDR7 is a member of the nuclear hormone receptor superfamily and acts as a ligand-inducible transcription factor regulating vitamin D-responsive genes.[21]The pathogenesis of AA is likely to be autoimmune andinflammatory. The ability of corticosteroids to abort AA suggeststhat the symptoms may be caused by inflammatory cytokines rather than another etiology. Another finding of our study wasthe elevation in CRP levels in subjects with AA (albeit a mildand non-statistically significant increase). This finding suggestsinflammatory mechanisms apart from the proposed immunemechanism.

Mansour et al., demonstrated 90% prevelance of vitamin D deficiency in apparently healthy hospital staff and health care professionals.[22]Zargaret al., found that 82% of healthy subjects had vitamin D defficiency.[23]Osullivanet al., and Al‑Kinidi reported a high prevalence of vitamin D deficiency in healthy Irish adults and Omani women,respectively.[24,25] Several conditions may contribute to low serum levels of vitamin D in the general population, including poor dietaryintake of vitamin D, sun avoidance and ⁄ or negligible sunexposure, possibly related also to impaired quality of life, andmalabsorption due to inflammatory bowel disease, gluten enteropathy, gastric surgery, biliary disease, or intestinalbacteria overgrowth.[26]In the current study, serum vitamin D level was significantlylower in AA cases when compared with healthy controls. Thiswas in agreement with previous similar studies.[6]This mayprovide evidence about the role of vitamin D deficiency in AApathogenesis.

In current study, there was significant negative correlation between 25(OH)D levels and the age of the patients. On the contrary, Yilmazet al. reported that there was no significant correlation between age and 25(OH)D levels in the studied groups.[6] The present study shows that 25(OH)D gradually declined from patchy AA to alopecia totalis/universalis. The least values were present in alopecia totalis/universalis.

The present work showed no significant association between 25(OH)D levels and gender of the studied case and lack of association between serum 25(OH)D level and disease recurrence or family history of AA. This study also show nonsignificant association between the duration of sun exposure/day and 25(OH)D levels.

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Conclusion

Serum 25(OH)D levels were negatively correlated with disease severity and pattern of hair loss. These data may provide evidence about the role of vitamin D deficiency in the pathogenesis of the disease. The present work showed no significant association between 25(OH)D levels and gender of the studied case and lack of association between serum 25(OH)D level and disease recurrence or family history of AA. This study also show nosignificant association between the duration of sun exposure/day and 25(OH)D levels.

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