### RESEARCH ARTICLE

# Evaluation of Serum Vitamin D levels in Vitiligo Patients- A Case Control Study from Tertiary Care Hospital in North India

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#### **ABSTRACT**

**Background:** Vitiligo is an acquired pigmentary condition characterized by white patches that affect the skin and the mucous membranes. Vitamin D is involved in the pathogenesis of various autoimmune conditions, including vitiligo. Its deficiency can be related to many such diseases and hence requires treatment with vitamin D analogues. We compared serum vitamin D levels of cases and controls in this case-control study.

**Objective:** To assess the serum Vitamin D levels in vitiligo patients attending the clinics.

**Material and Methods:** Seventy-two patients with vitiligo and the same number of healthy controls of age ranging from 18 to 60 years attending the outpatient department of our tertiary care centre were taken in the study, and the serum vitamin D levels were measured. Results were analyzed and compared with that of the control group. Statistical analysis was made to see the significance.

**Results:** In this study, there was a highly significant difference in the mean serum vitamin D level (p < 0.0001) between cases (17.51  $\pm$  12.31 ng/mL) and controls (28.01  $\pm$  15.84 ng/mL). We also compared the mean serum vitamin D level in cases and controls based on their demographic profile like age groups, gender, occupation, marital status, and in cases based on the disease-specific parameters like duration of disease, several patches involved, percentage of body surface area (BBA) involvement, type of vitiligo, progression of disease and family history of the disease. The mean level was decreased when these parameters were compared, but no strong statistical association was observed between the vitamin D levels and these parameters.

**Conclusion:** The results of this study show a difference in the mean level of serum vitamin D in the cases as compared to the controls. However, our study emphasizes that more such studies have to be carried out to further explore the relationship of Vitamin D with vitiligo, which later may evolve as a possible therapeutic alternative.

Keywords: Case control, Study, vitamin D, Vitiligo, Vitiligo.

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# INTRODUCTION

Vitiligo is a chronic acquired disease caused by several complex factors that destroy cells producing melanin, ultimately leading to depigmented macules and patches. The word vitiligo originates from the Latin word 'vitium' meaning blemish.<sup>1</sup>

Vitiligo is the most common depigmenting disorder and its prevalence in the Indian population ranges from 0.25% and 4%.<sup>2</sup> It is an acquired condition that can affect all sexes, age groups, and races worldwide.<sup>3</sup> Although vitiligo can begin to appear at any age, and most cases have an age of onset below the 3<sup>rd</sup> decade.<sup>4</sup> Various hypotheses surrounding the etiopathogenesis of the disease have been put forward, but no

single theory has been proved to be consistently associated with the disease pathogenesis, and vitiligo continues to be a poorly understood disease. The active form of Vitamin D - 1,25-dihydroxyvitamin D3, a fat-soluble vitamin, has been implicated in controlling and regulating various immune mechanisms. Vitamin D levels are reduced in several autoimmune disorders, including vitiligo.<sup>5</sup>

# MATERIALS AND METHODS

STUDY AREA- The study included the patients of vitiligo who attended the Skin OPD at Maharishi Markandeshwar Institute of Medical Sciences and Research (MMIMSR), Mullana.

## **Study Population**

The study population comprised 72 cases of vitiligo and 72 age and sex-matched healthy controls who attended the dermatology OPD after assessing their eligibility according to selection criteria.

#### **Inclusion Criteria**

The cases included in the study were patients of either sex. Patients between 18 to 60 years. Patients with disease severity and duration are diagnosed clinically as vitiligo.

#### **Exclusion Criteria**

The cases excluded from the study were patients suffering from any other dermatological condition, pregnant and lactating females, and patients already on vitamin D therapy.

### **Controls**

Controls were the healthy age and sex-matched attendants of patients who visited the hospital.

#### METHODOLOGY

A properly well-explained consent was taken from all the study subjects. A thorough history and dermatological examination and systemic examination were made. The severity of the disease was assessed by BSA according to the rule of 9. Blood samples were collected from cases and controls, and Vitamin D levels were analyzed. Analysis of serum vitamin D levels was done by chemiluminescence immunoassay [CLIA] provided by Siemens and run on Siemens ADVIA

Centaur XP. Analysis was done to determine the association of serum vitamin D levels in vitiligo patients. After collecting the data, it was tabulated and analyzed using proper statistical methods to evaluate p-values. The results were interpreted as:

- Normal/Sufficient vitamin D levels = more than 30 ng/mL
- Insufficient vitamin D levels = 10–30 ng/mL
- Deficient vitamin D levels = less than 10 ng/mL

The present study was conducted on 72 cases of vitiligo and 72 age and sex-matched controls attending the outpatient department of Dermatology, Venerology and Leprosy at Maharishi Markandeshwar Institute of Medical Sciences and Research, Mullana. Serum levels of Vitamin D among vitiligo cases were assessed and compared with that of the controls. The following points were summarized as follows:

The mean age of cases was  $30.08 \pm 12.99$  years. Most of the cases, i.e., 41.66% (n=30), were young adults aged 18-24. Females outnumbered males in our study, the F:M ratio being 1.11:1. Around fifty-two percent (38 cases) in our study were married. Most of the cases, i.e., 40 (55.55%) were from an urban background. The maximum number of cases (56.94%, n=41) had vitiligo for more than two years duration and most cases (n=34, 47.22%) had vitiligo patches involving less than 10% BSA. In our study, vitiligo vulgaris was the most common type of vitiligo, i.e., only two patients (2.77%) had universal vitiligo in our study. Only fourteen of the patients (19.44%) had stable vitiligo with no new lesions since the last

### RESULTS

Table 1: Demographic data and Vitamin D levels in cases and controls

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Items	Mean serum vitamin D (Cases)	Mean serum vitamin D (Controls)	p value	
Age				
• <35 years (n=50)	$16.16 \pm 11.35$	$26.02 \pm 16.28$	0.144	
• >35 years (n=22)	$19.11 \pm 13.36$	$31.76 \pm 14.57$		
Gender				
• Male (n=34)	$17.58 \pm 13.22$	$28.27 \pm 15.22$	0.0029	
• Female (n=38)	$17.46 \pm 11.62$	$27.79 \pm 16.59$	0.0024	
Vitamin D				
• Mean $\pm$ SD (n=72)	$17.51 \pm 12.31$	$28.01 \pm 15.84$	< 0.0001	

Table 2: Serum 25-hydroxyvitamin D levels of the different subgroups of vitiligo patients

Items	Mean vitamin D level in cases	SD	p-value
Type of vitiligo			
• Segmental (n=11)	11.84	5.71	0.0969
• Non segmental (n=61)	18.54	12.92	
Duration of disease			
• <6 months (n=9)	20.08	14.48	
• >6 months (n=63)	17.15	11.99	0.05060
Family History			
• Present (n=13)	18.21	11.70	
• Absent (n=59)	17.36	12.54	0.8236
Leukotrichia			
• Present (n=15)	13.81	7.76	
• Absent (n=57)	18.49	13.14	0.1925

year. A positive family history of vitiligo was found in a total of 13 cases (18.05%) and 15 cases (20.83%) had Leukotrichia associated with vitiligo. The mean serum Vitamin D level of cases was 17.15 ± 12.31 ng/mL and that of controls was 28.01 ± 15.84 ng/mL. Thus, cases were observed to have a significantly lower mean level than the controls (p-value=<0.0001). The majority of the cases (51.38%) had an insufficient mean level of serum Vitamin D levels. Although males and females both had a decreased mean level of serum Vitamin D, the results were almost similar in both cases and controls. No significant correlation was found between the mean level Vitamin D and the duration of disease, the number of patches, stability of the disease and the type of vitiligo.

## **DISCUSSION**

A total of 72 patients of vitiligo who attended the outpatient department of dermatology, venerology and leprosy at Maharishi Markandeshwar Institute of Medical Sciences and Research, Mullana were included in the study along with 72 healthy age and sex-matched controls. In our study, the age of cases ranged from 18 to 60 years with a mean age of  $30.88 \pm 12.99$  years, while the mean age of controls was 32.29  $\pm$  12.60 years. The majority of the cases, i.e., 30 (41.66%) were young adults between 18 and 24 years age, followed by 16 cases (22.22%) in the age group of 25 to 31 years. This was in concordance with the study of Karagün E. et al., where the patient and control groups' mean age was  $30.96 \pm 10.57$  and  $31.45 \pm 8.33$  years respectively. There was a comparatively higher mean age observed in the cases and controls groups  $(37.6 \pm 17.2 \text{ years and } 36.2 \pm 15.8 \text{ years})$  in the study done by Deepthi V et al. A contrasting mean age value was seen in the study by Prakash D et al.8 with cases having a mean age of  $43.78 \pm 14.70$  years and in the study of Ramalingam R et al.<sup>9</sup> with a mean age of cases and controls to be  $40.43 \pm 19.21$  years and  $39.95 \pm 18.60$  years, respectively. Our study consisted of 34 (47.22%) males and 38 (52.77%) females with F:M ratio of 1.11:1. Controls were matched to include the same number of males and females. Females outnumbered the males in our study. This was in concordance with the study of Alshiyab DM et al. 10 in which the female: male ratio was 1.38:1. However, some studies showed more male cases than controls like-Karagün E. et al.<sup>6</sup> (M:F ratio was 1.27:1), Khurrum H et al.<sup>11</sup>, and Sanjay B et al. 12 (M:F ratio was 1.5:1 in both the studies). The majority of the cases in our study that is 28 (38.88%) were students while 20 (27.77%) were housewives and 6 cases (8.33%) each was skilled/semiskilled workers including occupations like teaching, driving, tailoring, service personnel, etc. and unskilled workers like laborers, shopkeepers, etc. In our study, 38 (52.77%) cases were married, and 34 (47.22%) were unmarried, which included 17 (23.61%) married males and 21 (29.16%) married females. In our study, most of the cases, i.e., 40 (55.55%), were from urban backgrounds, while the remaining 32 (44.44%) were from rural backgrounds. Most of the cases, i.e., 41(56.94%) in our study had a long duration of vitiligo for more than 2 years. Various other studies like Prakash D et al.8 and Saleh HMA et al.13 both calculated the

mean duration of the disease, and they also had similar observations with cases having a long duration of disease. In the present study, most of the cases- 36 (50%) presented with less than 5 patches of vitiligo. Almost half the number of cases (47.22%) had a BSA involvement of less than 10%, and only 7 cases (9.72%) had extensive involvement of BSA of more than 30%. This was in concordance with the results by Silverberg JI et al., 14 who noted the maximum number of cases (57.8%) with BSA in the range of 1 to 5%. However, contrasting observations were seen in the study by Amer AM et al., 15 where the maximum number of cases (52.4%) had a BSA in the range of 28–40%. Most common type of vitiligo in our study was vitiligo vulgaris n = 44 (61.11%). Two cases (2.77%) in our study had universal vitiligo, and a single case presented with mixed vitiligo (1.38%). Deepthi V et al. <sup>7</sup> also showed similar findings with Vitiligo Vulgaris as the most common type (44.1%). However, this was in contrast to the study by Sanjay B et al. 12 where focal vitiligo was the most common type with 11 cases (44%) followed by 9 (36%) cases of generalized vitiligo. In our study, 13 (18.05%) cases had a family history of vitiligo. Similar results were obtained from the study conducted by Khurrum H et al., 11 who showed a positive family history in 20% of the cases. But the study conducted by Karagün E et al. 6 showed only 2% of the cases with a positive family history. There were 15 cases (20.83%) who presented with leukotrichia in our study and Prakash D et al.8 demonstrated a similar value of 26.7% cases with the same. In our study, 14 (19.44%) patients had stable vitiligo while the remaining 58 (80.55%) cases had actively progressive lesions in the last one year. The study of Saleh HMA et al. 13 showed almost half (47.5%) of the cases with having a stable course of the disease. The mean Vitamin D levels in our study were categorized as follows deficient (<10 ng/mL), insufficient (10–30 ng/mL) and Normal/Sufficient (>30 ng/mL). Majority of the cases, i.e., 37 (51.38%) had insufficient levels of Vitamin D with a mean of  $16.50 \pm 4.92$  ng/mL. Similar results were seen in the study by Deepthi V et al., where around 55% of the cases were observed to have the mean serum Vitamin D level in the range of 10–30 ng/mL. The mean Vitamin D levels noted in our study amongst cases ranged from 4.2 ng/mL to 55 ng/mL with a mean of 17.51  $\pm$  12.31, which was significantly lower than controls. In the control group, the lowest value was 5.72 ng/mL, the highest value was 90.66 ng/mL and the mean was  $28.01 \pm 15.84$ . (p-value <0.0001). Hence there was a statistically significant difference in the mean Vitamin D levels in the cases and controls. In another case control study by Deepthi V et al., <sup>7</sup> the mean levels of cases (19.8  $\pm$  13.6 ng/mL) and of controls (29.5  $\pm$  17.1 ng/mL) with a p-value of <0.004 were in concordance with our study. Some other studies with similar results include Saleh HMA *et al.*  $^{13}$  (12 ± 4.1 ng/mL in cases vs 48.6 ± 23.3 ng/mL in controls) (p-value = 0.001) and Amer AM et al.  $^{15}$  $(17.3 \pm 5.3 \text{ ng/mL in cases and } 25.8 \pm 7.9 \text{ ng/mL in controls})$ (p-value = 0.006). However, in the study by Sanjay B et al.,  $^{12}$ patients' serum vitamin D levels ranged from 3 ng/mL to 27.68 ng/mL (mean  $13.17 \pm 5.71$ ), while in controls, the levels ranged from 3 ng/mL to 26.8 ng/mL (mean  $14.20 \pm 6.59$ ) and this was not statistically significant (p value=0.5575). Other studies with contrasting results included Prakash D et al. 8 where cases had a mean of  $19.31 \pm 3.623$  ng/mL and controls had a mean of  $20.93 \pm 5.634$  ng/mL. However, the mean level in cases was lower than that of controls, it was not statistically significant, the study by Ramalingam R et al. 9 where serum vitamin D level was found to be low in both groups and with no significant difference (25.32  $\pm$  9.92 ng/mL in cases and 24.59  $\pm$ 11.71 ng/mL in controls; p=0.652). Karagün E et al.<sup>6</sup> also noted the patients' serum vitamin D levels which ranged from 6 to 42 ng/mL (mean:  $12.04 \pm 8.84$ ) and in the control group ranged from 8 to 39 ng/mL (mean:  $12.91 \pm 6.08$ ) with a p-value = 0.570, showing a non-significant relation. The lowest mean Vitamin D levels in our study was found to be in the age group of 32–38 years (13.46  $\pm$  7.56 ng/ml) followed by in the age group of 53–60 years (15.15  $\pm$  16.14 ng/mL). The study by Khurrum H et al. 11 noted the mean serum vitamin D level in cases of less than 35 years of age to be  $12.3 \pm 8.7$  ng/mL and of more than 35 years to be  $16.5 \pm 10 \text{ ng/mL}$  (p-value= 0.01). In our study, the mean serum vitamin D levels of cases less than 35 years was  $16.16 \pm 11.35$  ng/mL vs those aged 35 years or above was  $19.11 \pm 13.36 \text{ ng/mL}$  (p-value = 0.3348), thus no statistical difference between the groups was observed in our study which was contrary to the study by Khurrum H et al. 11 Prakash D et al.8 noted a similar result with mean vitamin D level of cases below 35 years to be  $20.67 \pm 3.811$  ng/mL and more than 35 years to be  $18.63 \pm 3.388$  ng/mL, but no statistically significant correlation was found (p-value = 0.076). This was also in concordance with the study of Saleh HMA et al. 13 where the two groups showed no statistical significance (p-value 0.671). In our study, the mean vitamin D levels of the males (17.58 ng/mL) were similar to that of females amongst cases. The mean serum vitamin D levels in male and female cases were  $17.58 \pm 13.22 \text{ ng/mL}$  and  $17.46 \pm 11.62 \text{ ng/mL}$ , respectively. This concordance with Khurrum H et al. 11 study results where the male and female cases had Vitamin D levels of  $11.9 \pm 7.09$  ng/mL and  $15.9 \pm 11.46$  ng/mL, respectively. The mean values in the study by Prakash D et al. 8 showed male cases with mean Vitamin D levels of  $19.23 \pm 3.881$  ng/mL and female cases with  $19.42 \pm 3.339$  ng/mL with a p-value = 0.864. Cases in our study with a disease duration of less than 6 months (n = 9) had mean serum vitamin D level of  $20.08 \pm 14.48$  ng/mL and those with disease duration of more than 6 months (n = 63) had mean serum vitamin D level of  $17.15 \pm 11.99$  ng/mL. This was however not significant and no correlation was seen between the duration of disease and the mean Vitamin D levels (p value = 0.5060). Prakash D et al, 8 grouped the cases according to disease duration- One group with a duration of less than 5 years and another with a duration of more than 5 years. The mean levels were calculated and they also did not note any statistically significant difference between the two (p-value = 0.535). This was in contrast to the study by Deepthi V et al., where a significant correlation (p-value = 0.001) was noted with a duration of vitiligo. The mean serum Vitamin D level in the present study was insufficient irrespective of the BSA involvement and no correlation was found between

Vitamin D insufficiency and an increase in BSA of vitiligo. (p-value of cases with BSA <10% vs cases with BSA >30% was 0.2896). This was following the results by Khurrum H et al., 11 where the cases with BSA less than 10% had a mean value of  $14.5 \pm 10.2$  ng/mL and cases with BSA more than 10% had a mean value of  $15.2 \pm 8.8$  ng/mL (p-value = 0.81). The mean serum Vitamin D level in the cases having generalized vitiligo in our study was 19.97 ± 13.97 ng/mL and that in acrofacial type was 13.54 ± 7.36 ng/mL. A decreased level of vitamin D in all the types of vitiligo was observed in our study, with the least value observed in Mixed type (8.78 ng/mL), but no significant correlation was found between the type of vitiligo and vitamin D levels. This was in concordance with the results in the study by Khurrum H et al. 11 where no statistical significance was found between the mean serum vitamin D levels in the generalised type (13.5  $\pm$  9.2 ng/mL) and in acral type (12.2  $\pm$  8.7 ng/mL) (p-value = 0.82). Although both the types of vitiligo were shown to have below-normal levels of vitamin D, the Segmental vitiligo cases in our study had a comparatively reduced mean serum vitamin D levels than the cases with non-segmental vitiligo (11.84  $\pm$  5.71 ng/mL vs.  $18.54 \pm 12.92$  ng/mL), p value=0.0969. The result was, however not statistically significant. Around 18% of the cases in our study had a family history of vitiligo and their mean vitamin D levels came out to be  $18.21 \pm 11.70$  ng/mL. The difference in the mean serum vitamin D levels in cases having a positive family history was not significant to that of cases without a positive family history (p-value = 0.8236). The results of Khurrum H et al. 11 were in contrast with our study in which the mean serum vitamin D level in cases having a positive family history ( $16.4 \pm 12.6 \text{ ng/mL}$ ) was significantly more than cases with a negative family history (12.6  $\pm$  7.9 ng/mL) (p-value=0.04). The cases with Leukotrichia were observed to have a mean serum Vitamin D level of  $13.81 \pm 7.76$  ng/mL in our study and this was not statistically significant compared to those without leukotrichia (mean =  $18.49 \pm 13.14 \text{ ng/mL}$ ) (p-value = 0.1925). Mean serum Vitamin D levels were compared in the two groups- one having a stable disease and the other having an unstable disease. The mean levels in both groups were insufficient with the stable vitiligo cases had a mean level of  $19.09 \pm 13.97$  ng/mL and unstable cases with a The study by Khurrum H et al. 11 showed similar results with unstable stable cases having mean serum vitamin D level of  $14.1 \pm 9.5$  ng/mL and stable cases having levels of  $11.4 \pm 8.6 \text{ ng/mL}$ .

## **CONCLUSION**

Vitiligo is one of the most common pigmentary conditions that practitioners encounter in the dermatology OPD. Although various pathogenic mechanisms have been put forward to determine the precise cause of the disease, no single theory has been proved to be conclusive. Although India is a tropical country that receives a good amount of sunshine throughout the year, there appears to be a high prevalence of vitamin D deficiency which is further intensified by the vegetarian diet by the majority. The possible role of vitamin D in various

diseases, particularly some autoimmune diseases, has provoked various researchers to formulate a hypothesis regarding the role of vitamin D in vitiligo. The treatment of the disease and the response to it varies; hence the prognosis is unpredictable. Our study aimed to note any possible relationship between the Vitamin D levels amongst the vitiligo cases. In our hospital, a tertiary health center for dermatological care, we selected 72 cases of vitiligo and 72 age and sex-matched controls and assessed the serum levels of vitamin D among the two groups. The analysis showed a statistically significant difference in the mean values of the two groups compared. The rest of the parameters showed a decrease in the mean vitamin D levels, but it was not significant. The study showed a mean serum vitamin D deficiency in vitiligo cases compared to the controls. This draws attention to the need for more studies aimed at assessing the relationship of vitamin D levels with vitiligo cases. With further supporting evidence, vitamin D may evolve as a possible option for treating vitiligo.

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