

Association of Serum Vitamin D₃ Levels with the Severity of Acne Vulgaris in Adolescents: A Cross-sectional Study from Western Odisha, India

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ABSTRACT

Introduction: Vitamin D₃ plays an important role in the immune system, and its deficiency has been implicated in various skin diseases, including atopic dermatitis and psoriasis. Acne is a common inflammatory skin disease of adolescence and young adulthood that affects the face and upper trunk. The scarring caused by acne can have a significant psychological impact on patients and greatly affect their quality of life. However, the association between the severity of acne and vitamin D₃ levels remains unclear.

Aim: To assess the role of vitamin D₃ in the severity of acne vulgaris in adolescents.

Materials and Methods: This prospective cross-sectional study was conducted at the Department of Dermatology and Biochemistry, VIMSAR, Burla, Sambalpur, Odisha, India, from August 2022 to November 2022. Adolescents between the ages of 10 and 19 years, of either sex, with acne were clinically examined, and the severity of acne was evaluated using the Global Acne Grading System (GAGS) score. Serum vitamin D₃ levels were measured using a Chemiluminescence

Immunoassay (CLIA) analyser (Electra FA). Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) software version 21.0 (SPSS IBM Corporation, Armonk, New York), and a p-value <0.05 was considered statistically significant.

Results: A total of 150 clinically diagnosed cases of acne vulgaris patients were evaluated. The study included 73 males and 77 females. The mean age of males was 19.1±2.3 years, and for females, it was 18.2±2.4 years. Serum vitamin D₃ levels were lower in patients with severe acne (18.03 ng/mL) compared to those with moderate acne (23.57 ng/mL) and mild acne (28.02 ng/mL). The association between serum vitamin D₃ concentration and the different degrees of acne vulgaris was statistically significant (p<0.05). There was a strong negative correlation between severe acne and serum vitamin D₃ levels (r=-0.042, p-value=0.002).

Conclusion: Vitamin D₃ deficiency was more prevalent in patients with severe acne, and serum vitamin D₃ levels were inversely correlated with the severity of acne vulgaris.

Keywords: Inflammation, Papular and pustular lesions, Skin disease, Skin follicles

INTRODUCTION

Acne vulgaris is a common skin disorder that can present with both inflammatory and non-inflammatory lesions. It mainly occurs on the face but can also affect the upper arms, trunk, and back [1]. The worldwide prevalence of acne is around 75-95%, affecting all adolescents [2]. It is classified as mild, moderate, or severe based on the severity of the disease. Mild acne is characterised by closed and open clogged skin follicles (comedones) with a limited number of inflammatory lesions on the face. Moderate acne is characterised by papular and pustular lesions on the face, while severe acne is characterised by the presence of nodules and cysts, with widespread lesions on the trunk and face [3]. Severe acne is more likely to cause scarring, which can have a significant psychological impact on patients and affect their quality of life [4].

The exact cause of acne is not well-established, but multiple factors may contribute to its development. These include the blockage of pilosebaceous units under the influence of androgens, hyperkeratinisation, colonisation of follicles by *Cutibacterium acnes*, and local release of proinflammatory chemical mediators in the skin [5]. Genetic factors play a significant role in susceptibility to acne, accounting for approximately 80% of cases [6]. Vitamin D₃ plays an important role in calcium homeostasis and metabolism. It also regulates the immune system and the proliferation and differentiation of keratinocytes [7]. Vitamin D has antioxidant properties, and several studies have suggested a potential role for

vitamin D in the development of acne. Vitamin D receptors are found in human sebocytes, which regulate lipid and cytokine production. This suggests a possible association between vitamin D and acne development [8-10].

Given the lack of definitive information regarding the association between vitamin D₃ and acne vulgaris, the present study aims to evaluate the relationship between serum vitamin D₃ levels and the severity of acne vulgaris in patients. The secondary objectives of the study are to estimate serum vitamin D₃ levels and assess the role of vitamin D₃ in the severity of acne vulgaris in adolescents.

MATERIALS AND METHODS

The present prospective cross-sectional study was conducted at the Department of Dermatology and Biochemistry, VIMSAR, Burla, Sambalpur, Odisha, India, from August 2022 to November 2022, with a duration of three months. The study protocol was approved by the Institutional Ethical Committee, with approval number 162-2022/I-F-O/32, dated 05.08.2022, and informed consent was obtained from the parents of the study participants.

Inclusion criteria: All clinically diagnosed cases of acne vulgaris within the age groups of 10-19 years, of either sex, attending the Dermatology OPD within the study period were included as cases.

Exclusion criteria: Patients with chronic systemic disorders such as malignancy, chronic renal disease, heart diseases, tuberculosis, and other skin lesions like psoriasis, systemic lupus erythematosus,

scleroderma, type-1 diabetes mellitus, and thyroid disorders were excluded. Patients under corticosteroid therapy, vitamin D₃ therapy for rickets, osteomalacia, or fractures, those taking multivitamins, and those with a history of taking oral isotretinoin in the last six months were not included in the study group. A few patients (08 in number) who did not fulfill the above criteria were excluded from the study.

Sample size calculation: Sample size $(n) = (z_{1-\alpha/2})^2 (p) (q) / d^2$

n = Desired sample size

$Z_{1-\alpha/2}$ = Critical value and a standard value for the corresponding level of confidence. (At 95% CI or 5% level of significance (type-I error) it is 1.96)

P = Expected prevalence or based on previous research

q = 1 - p

d = Margin of error or precision

Study Procedure

Clinically evaluated age groups of 10-19 years, of either sex, at the Outpatient Department (OPD) of Dermatology were taken for study. Biochemical analysis was performed at the Department of Biochemistry, VIMSAR, Burla, Sambalpur, Odisha, India. The aim and steps of the study were clearly explained to each patient. The initial evaluation included a detailed family history of acne, duration of the disease, age of onset, past medical history and other relevant variables such as smoking and sunlight exposure. The severity of acne was assessed using the Global Acne Grading System (GAGS) score. According to the GAGS criteria, the total body surface is divided into six areas: forehead, cheeks, nose, chin, chest, and back. Each area is assigned a score based on the ratio between surface area and the density of pilosebaceous units. Mild acne is marked by a score of 1-18, moderate acne by 19-30, severe acne by 31-38, and very severe acne by >39 [11, 12].

Biochemical analysis: Serum vitamin D₃ analysis: After taking all aseptic precautions, 2 mL of venous blood was collected from the antecubital veins of the patients. The collected blood was centrifuged to separate the serum, and the estimation of vitamin D₃ was performed within 24 hours using a fully automated CLIA analyser (Electra FA), following the manufacturer's instructions [13]. The reference range for serum vitamin D₃ levels (ng/mL) is shown in [Table/Fig-1].

Serum vitamin D ₃ level	Reference range (ng/mL)
Deficient	0-10
Insufficient	10-30
Sufficient	30-100
Toxicity	>100

[Table/Fig-1]: Reference range of serum vitamin D₃ level (ng/mL) as per manufacturer's instructions.

In the present study, none of the subjects belonged to the deficient, sufficient, or toxic groups. However, all 150 acne vulgaris subjects had insufficient levels of vitamin D₃.

The anthropological parameters, for the calculation of Body Mass Index (BMI), the weight and height of the subjects were used. BMI was calculated as the body weight in kilograms divided by the height in meters squared. BMI was used to define overweight and obesity [14].

STATISTICAL ANALYSIS

The recorded data were entered, checked, and analysed using SPSS version 21.0 (SPSS IBM Corporation, Armonk, New York). An unpaired t-test was performed to analyse the relationship between two variables, and an Analysis of Variance (ANOVA) test was used to analyse more than two variables to assess the severity of the disease. A p-value <0.05 was considered significant. The correlation between serum vitamin D₃ and disease severity was

analysed using Pearson's correlation coefficient. Quantitative data were presented as mean ± Standard Deviation (SD), while qualitative data were presented as frequency and percentage.

RESULTS

The demographic characteristics of acne vulgaris patients are shown in [Table/Fig-2]. In the present study included 150 patients, of which 73 (48.66%) were males and 77 (51.33%) were females. The mean age for males was 19.1 ± 2.3 years, and for females, it was 18.2 ± 2.4 years. The mean age of onset of acne in both sexes was 14 ± 2 years. Family history of acne was positive in 66 (44%) males and 84 (56%) females. The majority of the subjects (68%) had a normal weight, while 20%, 5.33%, and 6.66% were overweight, underweight, and obese, respectively. Among the subjects, 56.66% were not using sunscreen. In terms of acne severity, 30% had mild acne, 36.66% had moderate acne, and 33.33% had severe acne, with the face being the most affected area (60%), followed by the chest (30%) and back (10%) according to the GAGS score [12], as shown in [Table/Fig-2]. The mean age and BMI were not statistically significant (p-value=0.085 and p-value=0.073, respectively), while gender was statistically significant (p<0.05).

Characteristics	Acne patients (N=150)	p-value
Age in years		
Male (Mean±SD)	19.1±2.3	0.085
Female (Mean±SD)	18.2±2.4	
Gender		
Male N (%)	73 (48.66%)	0.002*
Female N (%)	77 (51.33%)	
BMI (kg/m²) (WHO Indian standard)		
Normal or less (18.5 ≤24.9)	102 (68%)	0.073
Overweight (≥25-29.9)	30 (20%)	
Obese (≥30)	8 (5.33%)	
Under weight (≤18.5)	10 (6.66%)	
Sun exposure		
>2 hours per day N (%)	105 (70%)	NA
<2 hours per day N (%)	45 (30%)	
Using sunscreen		
No	85 (56.66%)	0.001*
Yes	65 (43.33%)	
Family history of acne N (%)		
Male	66 (44%)	0.002*
Female	84 (56%)	
Mean age of onset of diseases, years	14±2	NA
Site of acne		
Face N (%)	90 (60%)	0.041*
Chest N (%)	45 (30%)	
Back N (%)	15 (10%)	
Disease severity		
Mild N (%)	45 (30%)	0.032*
Moderate N (%)	55 (36.66%)	
Severe N (%)	50 (33.33%)	

[Table/Fig-2]: Demographic characteristics of study population.

*p-value <0.05 was considered statistically significant, NA: Not applied; WHO: World health organisation

The comparison between serum vitamin D₃ levels and gender is shown in [Table/Fig-3]. The mean vitamin D₃ levels in females were 20.7 ± 4.6 ng/mL and in males were 23.63 ± 4.92 ng/mL, which was significant (p-value=0.003).

The association of serum vitamin D₃ concentration with different degrees of acne vulgaris is shown in [Table/Fig-4] and was statistically significant (p-value=0.001). [Table/Fig-5] depicts the

Parameter	Acne patients Male (n=73)	Acne patients Female (n=77)	Unpaired t-test (p-value)
Serum vitamin D ₃ level (ng/mL) mean±SD	23.63±4.92	20.7±4.6	0.003*

[Table/Fig-3]: Comparison of serum vitamin D₃ level among gender.
*p-value <0.05 was considered statistically significant

correlation of vitamin D₃ levels with different degrees of acne vulgaris. The correlation coefficient (r) values for mild, moderate, and severe acne were 0.055, 0.173, and -0.042, respectively (p<0.05). Severe acne had a negative correlation with serum vitamin D₃ levels (r=-0.042, p-value=0.002).

Clinical pictures of moderate and severe acne vulgaris are shown in [Table/Fig-6,7].

Parameter	Acne patients mild (n=45)	Acne patients moderate (n=55)	Acne patients severe (n=50)	Unpaired t-test (p-value)
Serum vitamin D ₃ level (ng/mL) (mean±SD)	28.02±1.72	23.57±1.76	18.03±1.53	0.001*

[Table/Fig-4]: Association of serum vitamin D₃ level and acne vulgaris.
*p-value <0.05 was considered statistically significant

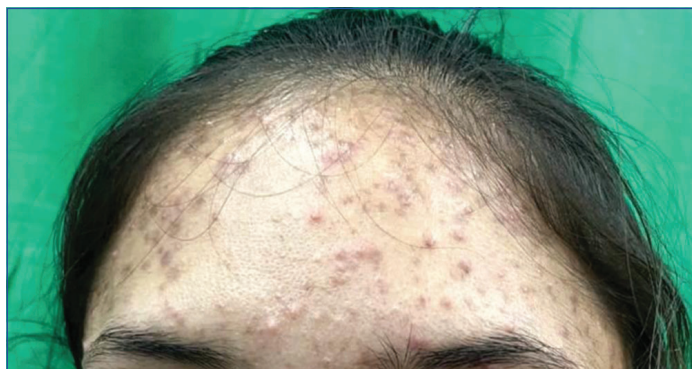
Degree of acne vulgaris	Serum vitamin D ₃ values	r-value	p-value
Mild (n=45)	28.02±1.72	0.055	0.01*
Moderate (n=55)	23.57±1.76	0.173	0.009*
Severe (n=50)	18.03±1.53	-0.042	0.002*

[Table/Fig-5]: Correlation of serum vitamin D₃ level with different degree of acne vulgaris.

*p-value <0.05 was considered statistically significant



[Table/Fig-6]: Photograph showing moderate acne vulgaris.



[Table/Fig-7]: Photograph showing severe acne vulgaris.

DISCUSSION

The most common form of acne is Acne vulgaris, which is characterised by a combination of inflammatory and non-inflammatory skin lesions, predominantly affecting adolescents [1]. Puspita F et al., have reported an association between vitamin D₃ and the severity of acne vulgaris. In their study, there was a higher prevalence of

acne in females (51.33%) [14]. The low Vitamin D₃ levels in severe acne may be attributed to psychological stress or inadequate time spent outdoors [15]. Hormonal effects during the menstrual cycle and puberty in females may contribute to the development of acne. Androgenic effects during puberty can lead to the enlargement of skin follicular glands and increased secretion of oily sebum [12]. In an environment rich in sebum, there is rapid growth of Cutibacterium acnes, leading to inflammation through activation of the innate immune system [16]. Previous studies have shown similar results [17].

There was no significant difference in the mean age of male and female patients (p-value=0.085). In this study, the patients were between the ages of 10-19 years, with a mean age of 14±2, which is consistent with a study by Alhetheli G et al., where the mean age of the study group was 16.55±4.99 years [17].

The study found that lower levels of vitamin D₃ were associated with more inflammatory lesions of acne, similar to a study by Lim SK et al., [5]. In the present study, 56.6% of the subjects were not using sunscreen, indicating a lower risk of vitamin D deficiency, which is consistent with a study by Neale RE et al., [18].

Female subjects with lower levels of vitamin D₃ had a higher prevalence of family history of acne in first-degree relatives compared to males (56% vs. 44%), as shown in [Table/Fig-2]. A similar study by Anaba EL and Oaku IR, found that family history was a risk factor, but not a cause, of acne severity in females [19].

The results showed that serum vitamin D₃ levels were higher in subjects who were exposed to the sun for more than 2 hours per day, accounting for 70% of the participants. This suggests that sun exposure enhances vitamin D₃ synthesis, consistent with a study by Alhetheli G et al., [17]. Humans primarily obtain vitamin D through the action of sunlight on the skin, converting 7-dehydrocholesterol into cholecalciferol [20].

In the present study, the majority of the subjects (68%) had a normal BMI level. This finding is consistent with studies by Lim SK et al., and Anaba EL and Oaku IR, where no significant association was found between obesity and Vitamin D₃ levels [5,19]. It has been observed that individuals with higher BMI tend to have higher fat content, which serves as a reservoir for fat-soluble Vitamin D₃. Approximately 10-12% of supplemented Vitamin D can be stored in adipose tissue, and the release of Vitamin D₃ from fat is slower compared to other tissues [21]. Therefore, excess body fat may lead to the sequestration of Vitamin D₃ and decrease its serum levels [22].

Serum Vitamin D₃ levels were found to be lower in severe cases of acne compared to moderate and mild cases, as shown in [Table/Fig-4]. Similar findings were reported in a previous study by Yildizgören MT et al., on nodulocystic acne, where relatively low serum Vitamin D₃ levels were observed [23]. Other studies by Puspita F et al., and Rosenstreich SJ et al., have demonstrated the anti-inflammatory and anticomedogenic effects of Vitamin D in acne patients. They found that Vitamin D can suppress the release of inflammatory cytokines such as IL-8 and IL-12, as well as the migration of Th1 and Th17 lymphocytes to the site of inflammation in acne vulgaris patients [14,24]. These factors may contribute to the severity of the disease.

There is also published evidence showing that Vitamin D can decrease T cell proliferation, B cell opsonising antibodies, and Th17 lymphocyte activities [25]. Additionally, Vitamin D exhibits antimicrobial effects by inducing antimicrobial peptide L37 in human sebocytes [26].

In the present study, a negative correlation was found between serum Vitamin D₃ levels and acne vulgaris severity. The correlations of Vitamin D₃ levels with different degrees of acne vulgaris are shown in [Table/Fig-5]. The correlation coefficient (r) value for severe acne was -0.042 at p-value <0.05. This finding is consistent with previous studies by Lim SK et al., and Puspita F et al., who

found a significant strong negative correlation between serum 25-hydroxy vitamin D 25-(OH)D levels and acne vulgaris severity, with correlation coefficients of -0.512 ($p < 0.001$) and -0.719 ($p < 0.05$), respectively [5,14]. Therefore, lower Vitamin D₃ levels are associated with greater severity of acne, highlighting the potential need for Vitamin D₃ supplementation in severe cases of adolescent acne.

Limitation(s)

The limitation of the present study was the power of the study. The power of actual sample size (158) was 0.83, and the power of the recruited sample size (150) was 0.81. Another limitation was the short duration of the study period, which was three months. Additionally, the patients who came for two days a week for three months were selected for the study. To validate the findings of the present study, further large-scale studies with longer durations should be undertaken.

CONCLUSION(S)

The present study found an inverse correlation between disease severity and vitamin D₃ levels, indicating the involvement of vitamin D₃ in the pathogenesis of acne vulgaris. Further studies are needed to evaluate the definitive role of vitamin D₃ in Acne so that serum vitamin D₃ estimation can be used as an early marker in acne patients and considered as an additional therapy in the management of Acne vulgaris in Dermatology.

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