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Status of vitamin D level in newly diagnosed pemphigus vulgaris patients: A case–control study from tertiary care centre of Eastern India

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***Abstract*---**Background: Pemphigus vulgaris (PV), one common type of pemphigus, that affects the skin and mucous membranes, produce keratinocyte acantholysis by desmoglein-3 autoantibodies. Many medical conditions, including autoimmune illnesses, have been linked to vitamin D deficiency. Aim of study: To measure vitamin D level of Pemphigus Vulgaris patients and compare that with healthy controls& severity of disease. Material and Methods: Newly diagnosed PV patients (44) were compared to 50 healthy controls in this case-control study on vitamin D levels. At S.C.B. Medical College & Hospital, Odisha, the dermatology department chose all patients over a two-year period (2017-2019). Pemphigus vulgaris lesion severity ratings were used to measure the disease severity. The 25(OH) D levels in the

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blood were determined using the ECLIA method (Electro chemiluminescence immunoassay) in Cobas e411 analyzer. Analyses of the data included independent t-tests and pearson correlations. Results: Gender, age, and other characteristics of both groups were virtually identical. The study comprised 44 patients (27 females and 17 males) and 50 controls (including 19 men and 31 women). Eighty-six percent of patients had moderate to severe involvement, whereas 11% had minor lesions. Patients had Vitamin D levels of 14.44±9.03, compared to 32.88±15.66 for controls ($p<0.05$). There was a correlation between vitamin D levels ($p<0.05$) and the severity of the disease. Conclusion: Patients with Pemphigus Vulgaris exhibited considerably lower levels of vitamin D in their serum than healthy individuals, which could contribute to the worsening of their disease. Pemphigus patients should be aware of the importance of enhancing their vitamin D levels. Serum Vitamin D levels were found to be a significant predictor of disease severity.

***Keywords*---**Pemphigus vulgaris, Vitamin D, Calcitriol, Autoimmune disease.

Introduction

Bullous diseases caused by autoantibodies against desmosomal proteins are known as pemphigus[1]. Pemphigus vulgaris (PV), the severe and prevalent type of Pemphigus presents with symptoms of blisters&ulceration on the skin and/or mucosa, as well as a possible deadly outcome[2].Even though PV's incidence ranges from 0.5 to 4 cases per 100000 person-years, geographic location has a considerable impact on the prevalence, with around 1 in every 100,000 persons in Iran, the Mediterranean area, the Middle East, and India having the greatest incidence rate [3, 4]. Despite the fact that the underlying causes of PV remain a mystery, it is known that the disease has an immunological base. Acantholysis of keratinocytes, which results in clefts or bullae, is thought to be caused by autoantibodies against the desmosomal component desmoglein-3 [5]. There is speculation that environmental and genetic variables in addition to Autoantibodies, may have a role in the underlying process of PV due to non apparent diseases in some patients even with Autoantibodies in blood[6,7].All of the above conditions have been connected with low vitamin D levels as a part of the ecosystem [8, 9, 10]. Vitamin D's immunomodulatory capabilities have long been recognised as a means of reducing the risk of autoimmune disorder and enhancing the body's natural defences against infection [10, 11]. The 1,25 (OH)2 D and vitamin D receptor (VDR), which mediates the vitamin's genomic activity, can be produced by immune cells [12]. T/B lymphocytes, dendritic cells, monocytes/macrophages, and other immune cells are also at risk. Its epidermal expression connects the environment to the immune system by increasing CD4+ CD25+ regulatory T cells, which in turn modulates cutaneous immunological responses[13]. As a result, Calcitriol (1,25dihydroxy Vitamin D) directly inhibits Th1 cytokines and promotes Th2 cytokines in decreasing autoimmune reactions[14].

In this study PV patients and healthy controls were compared for vitamin D status because of wide spectrum of immunomodulatory potentials of 1,25-dihydroxy Vitamin D & Insufficient data about vitamin D deficiency in PV patients.

Objectives

Compare Vitamin D levels between Pemphigus Vulgaris patients and healthy controls. To establish a connection between vitamin D levels and illness severity.

Material and Methods

An eastern Indian tertiary care hospital's Dermatology Department(Sri Ram Chandra Bhanj Medical College &Hospital, Cuttack) conducted this clinical study from June 2017 to June 2019. The study was given the go light by the college's ethical committee.

Inclusion Criteria

A clinical and histological (suprabasal cleft and acantholysis) diagnosis of PV [12]. Recently diagnosed PV Patients, not on systemic corticosteroids or other treatments. Healthy people recruited from volunteers without any autoimmune or inflammatory disorders, that matched the patients' age, sex.

Exclusion Criteria

Supplements, contraceptives, or steroids used by PV patients. Renal or hepatic disease in PV patients. Healthy controls using Vitamins, corticosteroids, contraceptives, or suffering from heart disease or diabetic.

Data Collection

Using simple sampling procedure, we were able to include every patient who was admitted to the hospital and met our criteria. Effect of seasonal variation of Vitamin D was omitted as the study was done over a period of 2years. All patients and controls were given the opportunity to provide informed consent. Inclusion and Exclusion Criteria were used to identify patients and controls for the study. Observer bias due to inter-individual variability was reduced by having all patients assessed by a single dermatologist. To learn more about each patient's medical history and the medications and supplements they were taking, we conducted interviews with both the patients and the controls. Nil, Mild, Moderate, and Severe[15] were the classifications used to identify the severity of skin and oral mucosa involvement.

After 12- to 14-hour fasting, the antecubital vein of each participant was utilised to collect blood samples (5 cc) in the morning, which were then centrifuged at 3000 rpm for ten minutes at 4˚C. In order to conduct further biochemical tests, the plasma was immediately frozen at -80°C and stored. "The ECLIA method, in Cobase411 analyzer was used to measure 25(OH)D levels. Samples having a serum level of above 30ng/ml were considered normal[16]. Samples with serum

levels between 10 ng/ml and 30 ng/ml were considered insufficient, while those with serum levels below 10 ng/ml were deemed deficient[16].

Stastical Analysis

Study Structure**:**
No. of Sample used – 94 (Case-44 & Control-50)
Types of variable – Numerical and Categorical
Number of group – 2 groups and >2 variables
Study design – Unpaired/Independent (observational)
Distribution – Normal

Methods
It is an observational study (case-control) and randomized simple sampling method was used. Sample size calculated using the Population (N). Confidence level is 95% and z score is 1.96.

Data Analysis

SPSS version 22 and Microsoft Excel were used for data analysis. Descriptive statistics were done for demographic variables and presented with plot and chart. Data in non-normal form was given in the form of n and percent. P value less than or 0.05 is deemed statistically significant.
Tests used:

1. Unpaired t-test (for normal data)
2. Pearson Correlation

Results

For this study, we enrolled 44 patients who qualified for inclusion (27 females and 17 men, ages 24-53 years). All of them completed the trial. There were no gaps in our data, and statistical analysis included all measurements. Fifty healthy persons were served as controls (19-males,31-females,aged 24-63yrs). At the outset, we checked for potential confounding variables like gender and age distribution and found no significant differences across the groups. [Table: 1].

Table:1- Age, Gender indices of patients & controls(Demographic data)

	Patients (n=44)	Control (n=50)	p value
Age (years)	41.88±7.25	46.7±10.37	0.62
Sex (M/F)	17/21	19/31	0.089

Of 44 cases 29% have serum vitamin D level(>30ng/ml) and 71% have ≤30ng/ml [Fig: 1].

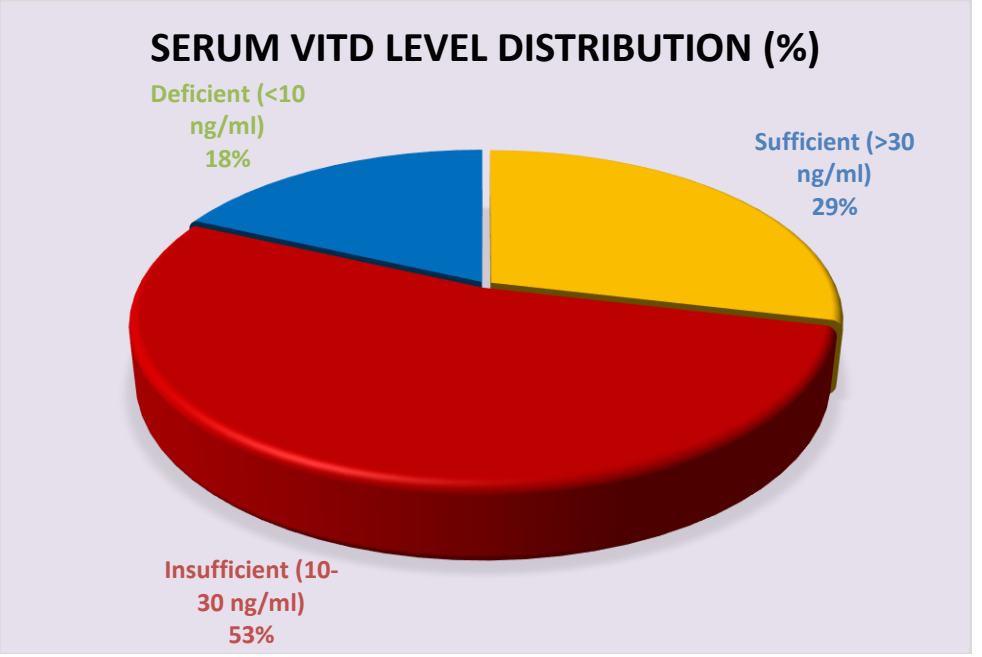


Fig: 1 -Serum Vitamin D Level distribution

Patients had vitamin D levels of 14.44±9.03, compared to 32.88±15.56 in the control group. With a p value = 0.001, this discrepancy was statically important[Table: 2].

Table:-2-Vit. D status in PV patients & Control subjects

	Group	Number	Mean (Vit. D Level)	S.D. (Vit. D Level)	Mean Difference with 95% CI	P Value
Serum VitD (ng/ml)	Patients	44	14.44	9.03	-18.57 (-23.60,-13.54)	0.046
	Controls	50	32.88	15.56		

Unpaired t-test

A standard deviation (SD) of 9.09 ng/mL was found in female patients' vitamin D levels, whereas the SD was 15.99 ng/mL in female controls. Male patients had a mean vitamin D level of 14.43±9.01, whereas controls had a level of 30.34±16.08.p-values of ≤0.05 indicated statistical significance for the two studies, which were both statistically significant. [Table:3].

Table: 3 Vitamin D level based on Gender

Gender		Case	Control	Total	P value
Female	Number	27	31	58	0.036
	Mean	14.68	34.16	24.31	
	S.D.	9.09	15.99	16.33	
Male	Number	17	19	36	0.047

	Mean	14.43	3.34	24.24	
	S.D.	9.01	16.08	15.86	
Total	Number	44	50	94	0.046
	Mean	14.44	32.88	24.25	
	S.D.	9.03	15.56	15.91	

p value represented by unpaired t-test

When it came to vitamin D levels gender wise, there was no discernible gender difference among patients (male ~ female) .The p-value was more than 0.05[Table-4]

Table: 4 Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Male_Case - Female_Case	4.64918	11.89388	2.88469	-1.46609	10.76444	1.612	16	.012
Pair 2	Male_Control - Female_Control	-.42789	25.15230	5.77033	-12.55092	11.69513	-.074	18	.042

P value is < 0.05, shows significance of the study.

Eleven patients had severe illness (including the skin and mucosal), twenty seven had moderate disease, and six had mild disease. 86.3% of the patients were diagnosed with a moderate or serious illness. Mean vitamin D levels differed by illness severity, with a p-value of 0.004 in this study [Table:5].

Table:-5 Vitamin D levels bases on severity

Severity	Number	Mean (Vit D Level)	S.D	P value
Mild	5	20.15	8.74	0.004
Moderate	27	14.27	9.01	
Severe	11	14.06	10.19	

It is found out from pearson correlation test that vit. D level in case & control have pearson correlation coefficient =0.240, which shows they are weakly correlated to each other [Table: 6].

Table: 6 Paired Samples Correlations

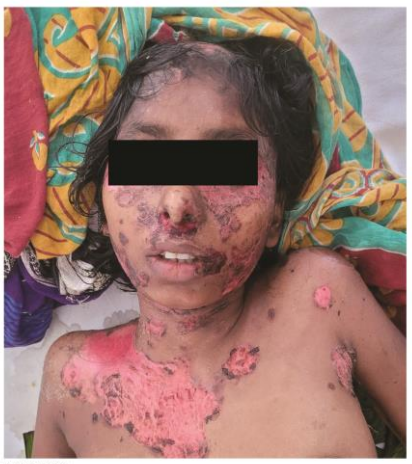
		N	Correlation	Sig.
Pair 1	Case_VitD_Level & Control_VitD_Level	44	.246	.007

Pearson correlation test

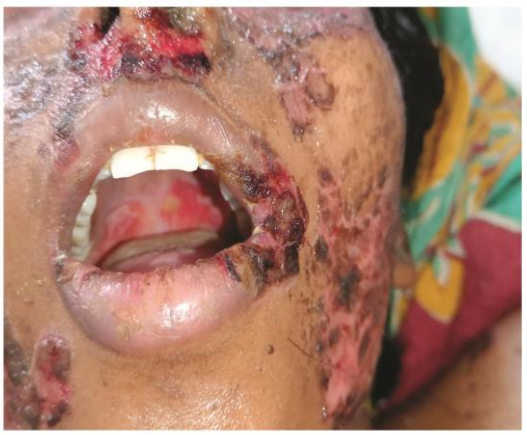
Discussion

Hypersensitivity and ulcerations are characteristic of the life-threatening autoimmune disease known as pemphigus vulgaris [Photograph: 1,2].Serum vitamin D levels in PV Cases who were not taking medication were substantially lower than in healthy controls($p<0.05$) as shown in our study. Vitamin D deficiency may increase the risk of Pemphigus vulgaris, according this study also. We found many studies, where vitamin D levels were lower in PV patients than in healthy controls [1,7,17,18,19]. This reduced vitamin D level was not associated with age, body mass index (BMI), or sun exposure pattern, according to a study by EL-Komy et al. that looked at the vitamin D status of 34 people with pemphigus [17]. There was a negative correlation between the severity of the disease(oral mucosa) and vitamin D levels in PV patients, according to Zarei et al.[1].Another literature by Marzano AV etalalso shown similar low vitamin D level as our study[20].

Contrary results have also been reported so far from various studies regarding the relationship between vitamin D deficiency and PV[7,17,19,21]. Researchers found vitamin D deficiencies in both PV patients and healthy controls in a research by Joshi et al. of 30 patients from the North Indian population with PV [7], similar to the findings of Moravvej et al. [18]. Reduced TGF-β and IL-17 ratios in PV patients suggest that T cell activities are out of whack [7]. Research conducted by Marzano et al. on 67 patients [21] found that patients with pemphigus vulgaris and bullous pemphigoid had lower 25 OH vitamin D levels, and that those with severe hypovitaminosis D were more common. Patients with bullous pemphigoid and healthy controls had no difference in vitamin D blood levels, according to Tukaj et al [19]. A possible function for vitamin D deficiency in Pemphigus vulgaris onset has been suggested by the research discussed above.
Our analysis indicated a significant correlation between PV severity and vitamin D deficiency ($p<0.005$), which is consistent with a recent study that found a relationship between PV and oral disease severity[1]. There are some studies where no significant correlation were found[16,22]. In gender wise analysis it is found that mean vitamin D level was similar ,which showed equal exposure of male,females to sunlight due to work pattern.



Photograph-1
Pemphigus vulgaris Showing Skin Lesions on head, Face, Chest



Photograph-2 PV Showing Mucosal Lesions

Conclusion

Those newly diagnosed with pemphigus vulgaris who have not had any treatment have significantly lower blood vitamin D levels than healthy controls, according to the findings of this study". The severity of the disease was reported to be correlated to vitamin D levels. Maintaining proper levels of serum vitamin D can help to prevent and treat Pemphigus vulgaris. For patients with Pemphigus vulgaris, vitamin D levels should be regularly checked, and supplements should be given as needed. Additional well-conducted clinical trials and cohort studies with a larger number of participants are required to determine whether vitamin D has a causal role and to pinpoint its exact effects in pemphigus vulgaris, even though no causality can be demonstrated from this study.

Conflict of Interest: Nil

Source of Support: Nil

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