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Assessment of serum vitamin D level in patients with cutaneous warts: a case-control study

Azadeh Goodarzi, MD [1*]
Mahsa Farshidzadeh, MD [2]
Masoumeh Roohaninasab, MD [1]
Najmolsadat Atefi, MD [1]
Elham Behrangi, MD [1]

1. Department of Dermatology, Rasoul Akram Hospital, Iran University of Medical Sciences, Tehran, Iran
2. Department of General Medicine, Rasoul Akram Hospital, Iran University of Medical Sciences, Tehran, Iran

**Corresponding authors:*
Azadeh Goodarzi , MD Department of Dermatology, Rasoul Akram Hospital, Iran University of Medical Sciences, Tehran, Iran
Tel: +98 9123882448
Email: Goodarzi.a@iums.ac.ir; A.Goodarzi.Dermatology@gmail.com

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Background: The use of vitamin D and its analogues in the treatment of warts has been to be effective and painless without any considerable side effects, unlike some other routine safe and effective therapeutic modalities such as cryotherapy that in some cases is really difficult to perform due to related pain, especially in the children. Owing to a probable relationship between the deficiency of serum vitamin D and warts, this research aimed to find the association between level of serum vitamin D and warts.

Methods: This case-control study was conducted on 56 subjects in Rasoul Akram Hospital, Tehran, Iran, for 18 months. The subjects were randomly divided into case and control groups, including 28 age and sex-matched subjects in each of them. The required data were extracted through a questionnaire. Finally, the data were analyzed in the SPSS software V.22 using tests such as Mann-Whitney and Pearson correlation.

Results: In this study, 56 people were examined, and 28 subjects were included in both case and control groups. The mean serum level of vitamin D in both case and control groups was 23.564 and 31.593, respectively. The association between serum levels of vitamin D in the group with and without wart was not significant in both men and female groups. Serum vitamin D levels were compared in two groups of under 18 years old and between 18 and 50 years old, which were not statistically significant.

Conclusion: According to our results, there is no significant relationship between serum vitamin D levels and existence of warts. This relationship is not statistically significant at the level of age and sex variables, and there is no difference in age and gender between the case and control groups.

Keywords: wart, human papillomavirus, vitamin D, gender, age

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INTRODUCTION

Warts are common skin lesions, which are solid, skin-colored or gray-brown bumps with a rough surface and a few millimeters of thickness. They can be single or multiple. In addition, if they join, they can make plaque and regular shapes on a rough surface [1]. Some kinds of them are all over the body and have a larger size and amount than those of normal warts [2]. They are usually painless. If they become dense or exist around the joints and nails, they can be painful. Sometimes, their surface is covered with full of fissures; therefore, it can cause pain [1].

Formation of warts is the most common effect of human papillomavirus (HPV). HPV is a small duplex virus having more than 100 types [3]. It causes warts after infection of squamous epithelial

cells and results in DNA replication inside them [4].

The tumor is made by polymorphic viruses and can be created in various parts of the body, such as the skin of the hands and feet, the mucosa and the skin of the genital, larynx and oral mucosa. However, virus replication occurs in areas where keratinocytes are quite distinct, such as the spinosum, granular and basal layer of squamous epithelium [3]. First, it targets these cells and replicates there in a hidden form. With this replication, it can cause hyperplasia and hyperkeratosis all over the body [2].

No specific information is available on the incidence of non-genital warts, but studies have revealed that it is more prevalent in children and adolescents [5]. In 2013, one study in the United States reported a prevalence of wart among person from birth to 17; the highest one was 6.8% in children aged 9 to 11 [6].

Additionally, two population-based studies reported the prevalence of wart to be 84.1% in the United States and 12.9% in Russia. Other studies reported the prevalence of 12% in children aged 6-4 years in the United Kingdom, and 24% in adolescents aged 18-16 in Australia [5].

No proven treatment exists for wart diseases. Most HPV infections can be treated with special tools having direct destructive effects on lesions, including cryotherapy [7,8]. All of these requested methods are used to treat warts, but they are painful, expensive and time-consuming, and none of them is the gold standard [9]. One of the newest method is the use of vitamin D [10]. Several studies have investigated the association between vitamin D deficiency and bacterial and viral infectious diseases. The effect of vitamin D on the treatment of infectious diseases was also studied [11]. In 2011, a successful treatment of warts by topical active vitamin D in a patient with a transplanted kidney was reported [12]. In another case report, a successful treatment of anogenital warts was reported in a newborn with topical active vitamin D [13].

In one study on 21 patients with warts, intralesional injection of vitamin D eventually caused 81% of patients to show complete disappearance of warts. For this reason, intralesional injection of vitamin D was proposed as an alternative treatment modality [9]. Intralesional injection of an immunotherapy agent into warts can stimulate the immune system against HPV cells. Vitamin D regulates the proliferation and differentiation of epidermal cells. It has a receptor on melanocytes, keratinocytes, fibroblasts and immune cells. Therefore, it enhances cytokine production [12]. In addition, EI-Taweel *et al.* showed that Vitamin D could be successfully used in topical and injectable forms [14].

According to various studies, the use of vitamin D and its analogues in the treatment of warts has been effective, but treatments like cryotherapy are difficult to perform. In addition, considering the higher prevalence of skin warts in children and the pain concerns of treatments in this age group, finding a safe and non-invasive therapy is necessary. Therefore, due to a probable relationship between the deficiency of serum vitamin D and warts, we aimed to design a case control study to investigate the association between the level of serum vitamin D and warts; so if it is found any relationship between vitamin D level deficiencies and wart, hypothetically correction for a normal range and/or use of vitamin D analogues could be a potential protective strategy or adjuvant therapy for warts. To conduct this study precisely, and owing to the importance of demographic variables, we examine this relationship in different sexes and age groups.

MATERIALS AND METHODS

Participants and study design

This case-control study was conducted on 56 subjects in Rasoul Akram Hospital, Tehran, Iran, for 18 months. This study had case and control groups, including 28 subjects in each of them. Furthermore, the participants were age-matched in two groups (under 18 years old as children and 18-50 years old as adults). According to gender, they were also matched in both case and control groups. The case group was selected from patients with wart referred to the Dermatology Clinic of the hospital, based on clinical examinations and diagnostic tests of warts. Moreover, the control group was randomly selected from staffs of the hospital. Inclusion criteria were the age, gender, diet, physical activity and sun exposure in the normal range of the patients.

Data collection methods

After finalizing the subjects, based on these variables, the required data were extracted through a questionnaire. It contains some questions about

age, sex, number of warts, involvement area, amount of daily activity and outside exercise, diet, usage of food supplements, sun exposure and vitamin D intake. In addition, the patient was requested to test vitamin D levels. The hospital's laboratory measured this amount using the ELISA technique. In the process of blood sampling, **5** cc blood was taken from the patients. This process is performed in accordance with the principles of safety and health. After bleeding, the patient is placed in a sitting position for 5 to 10 minutes, and then left with a good general condition. Finally, laboratory reports were recorded on a checklist.

Ethical considerations

In all steps taken in this study, the principles of the Declaration of Helsinki (ethical principles for medical research involving human subjects) and Iran University of Medical Sciences Ethics Committee were followed (IR.IUMS.FMD.REC 1396.8921215072). The study was approved by Iran University's Ethics Committee. The process of blood sampling from the patients was performed with the written informed consent.

Statistical methods

The data were entered into the statistical analysis software, SPSS, version 22, and were then statistically analyzed. The results for the quantitative variables were reported in Mean ± SD format, and the ordinal qualitative variables were reported in percentages. The Mann-Whitney U test was used

Table 1. Demographic data of participants (Case: patients with wart, Control: matched individuals without wart).

Variables	Group	Frequency	Percentage%	*P*-Value
Gender	Male	Case= 15	53.6	0.450
		Control=12	42.9	
	Female	Case=13	46.6	0.450
		Control= 16	57.1	
Age group	Case	Less than 18=12	42.9	0.450
		18-50=16	57.1	
	Control	Less than 18=12	42.9	0.450
		18-50=16	57.1	
Daily activity				
Case	Low	2	7.1	0.001
	Medium	20	71.4	
	High	6	21.4	
Control	Medium	25	89.3	0.001
	High	3	10.7	
Diet with dairy products				
Case	Low	5	17.9	0.001
	Medium	21	75	
	High	2	7.1	
Control	Low	3	10.7	0.001
	Medium	28	89.3	
Sunscreen use				
Case	Yes	22	78.6	0.221
	No	6	21.4	
Control	Yes	17	60.7	0.023
	No	11	39.3	
Sun exposure				
Case	Low	7	25	0.001
	Medium	17	60.7	
	High	4	14.3	
Control	Low	4	14.3	0.007
	Medium	24	85.7	
Wart	Less than 5	20	71.4	0.696
	More than 5	8	28.6	

to compare quantitative variables, and the chi-square test was used to compare qualitative ones. Furthermore, a correlation between quantitative variables was investigated using Pearson correlation and spearman rank correlation tests. The value less than 0.05 was considered statistically significant.

RESULTS

In this study, 56 people were examined, and 28 subjects were included in both case and control groups. The age of the participants was in the range of 5 to 46. The mean age in the case and control groups was 24.67 ± 13.61 and 25.36 ± 13.59, respectively. The patients were age-matched in the two groups, under 18 with 12 participants and 18-50 years old with 16. All demographic information for both case and control groups are summarized in Table 1. Furthermore, the mean serum level of vitamin D in both case and control groups was 23.564 and 31.593, respectively (Table 2). The serum level of vitamin D in the case group does not follow the normal curve in our study (Figure 1).

The amount of vitamin D levels was divided into three levels, which the highest percentage was approximately the insufficient level with 53.6% and 46.4% in both case and control groups, respectively (Table 3).

Furthermore, the number of warts in the case group was determined in two levels. First, one was less than five with 71.4% in 20 individuals, and the second one was above five with 28.6% in eight individuals. Additionally, the association between serum vitamin D level and warts was not statistically significant (P = 0.696).

Daily activity in both case and control groups was the highest in the middle level with 71.4% and 89.3%, respectively. According to the uses of sunscreen, 21.4% in the case group and 39.3% in the control group use it. Other information collected is sun exposure, divided into three levels: low, moderate, and high. In both case and control groups, the average level was 60.7% and 85.7%, respectively.

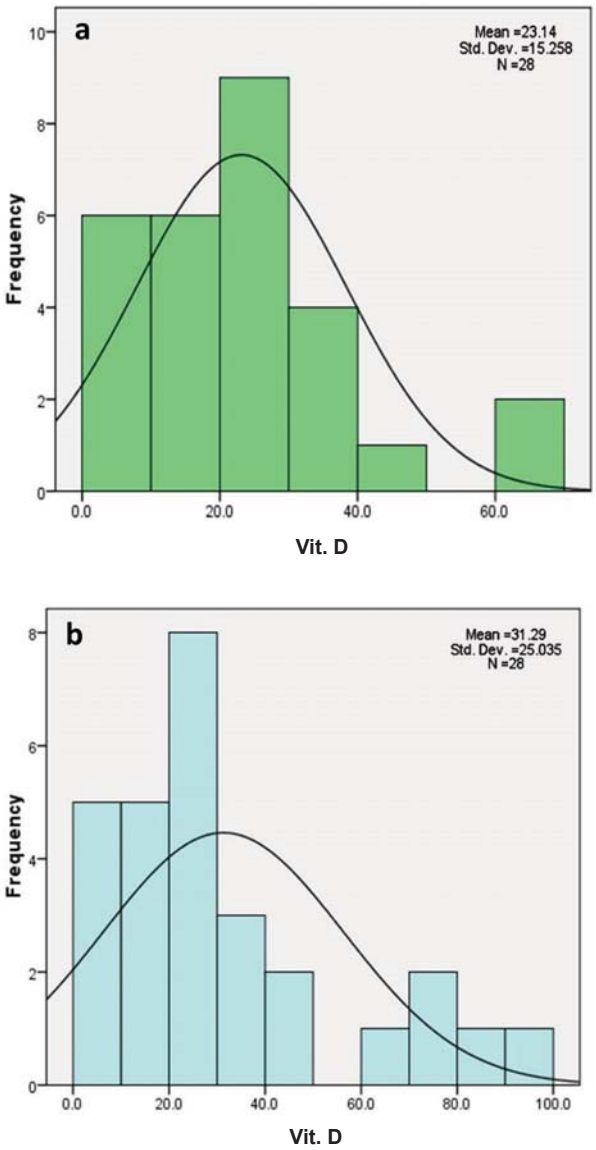

Figure 1. Serum vitamin D level in the case (a) and control (b) groups.

The dairy diet is also ranked again at three levels: low, middle and high. In the case group, it was 75% at the middle level, and in the control group, the highest was 89.3% again in the middle group.

Furthermore, the rate of involvement in different parts of the body was examined. Hands had

Table 2. Mean serum levels of vitamin D in the case and control groups.

	Vit. D Value (ng/ml)						
Group	**Mean**	**Median**	**Mode**	**Standard Deviation**	**Variance**	**Minimum**	**Maximum**
Case	23.564	21.4	2.7	15.127	228.83	2.7	67
Control	31.593	22.35	7.3	24.841	617.07	7.3	90

Vitamin: Vit

Table 3. Vitamin D levels status in the case and control groups.

		VitD_Range			
Case and Control	**Vit D level**	**Frequency**	**Percentage**	**Valid Percent**	**Cumulative Percent**
Case					
Valid					
Deficient	<10	6	21.4	21.4	21.4
Insufficient	10<D<30	15	53.6	53.6	75.0
Sufficient	D>30	7	25.0	25.0	100.0
Total		28	100.0	100.0	
Control					
Valid					
Deficient	<10	5	17.9	17.9	17.9
Insufficient	10<D<30	13	46.4	46.4	64.3
Sufficient	D>30	10	35.7	35.7	100.0
Total	28	100.0	100.0		

the highest percentage with 39.3% involvement (Figure 2). The serum level of vitamin D was determined by gender in patients with warts (case group). The average in both male and female groups was 22.307 and 25.015, respectively (Table 4). Then, the serum level of vitamin D in the groups with and without warts were compared to each other, and the association was not significant in both male and female groups with $P = 0.435$ and with $P = 0.726$, respectively (Table 5). In both case and control groups, the association between serum vitamin D level and age was measured, which was not statistically significant in both groups ($P = 0.658$, $P = 0.107$, respectively) (Table 6).

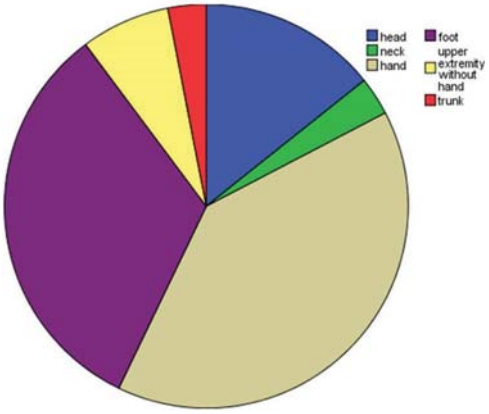

Figure 2. The prevalence rate of warts in different parts of the body.

Table 4. Serum level of vitamin D in case and control groups in terms of gender.

Group/Gender	**Mean**	**Std. deviation**	**Variance**	**Median**	**Minimum**	**Maximum**
Case						
Male	22.307	16.499	272.246	18.60	2.7	67
Female	25.015	13.892	193.006	22.90	5.4	60
Control						
Male	27.433	19.53	381.446	21.65	8.9	77
Female	34.712	28.40	806.789	22.65	7.3	90

Standard, Std.

Table 5. Mean serum levels of vitamin D with respect to gender in cases with and without warts.

		Ranks			
Gender	**Group (Case and Control)**	**N**	**Mean rank**	**Sum of ranks**	***P*-value**
Male	Vit. D				
	Case	15	12.93	194.00	
	Control	12	15.33	184.00	0.435
	Total	27			
Female	Vit. D				
	Case	13	14.38	187.00	
	Control	16	15.50	248.00	0.726
	Total	29			

Number, N; vitamin, Vit

Table 6. Vitamin D level status in case and control groups according to age groups (children and adults).

Group	Age Group		Total
	Less than 18	Between 18 to 50	
Case (P=0.658)			
Vit. D range			
Deficient	2	4	6
Insufficient	6	9	15
Sufficient	4	3	7
Total	12	16	28
Control (P=0.107)			
Vit. D range			
Deficient	2	3	5
Insufficient	4	9	13
Sufficient	6	4	10
Total	12	16	28

In the case group, serum vitamin D levels were compared in two groups of under 18 years old and between 18 and 50 years old, which were not statistically significant (P = 0.164). In addition, this comparison was made in the control group, which did not show a significant correlation (P = 0.246).

DISCUSSION

Aktas *et al.* conducted a study to evaluate the effect of vitamin D injection on the treatment of warts. The results of their study showed that injections of vitamin D into the lesion could be effective in the treatment of warts [9]. Moscarelli *et al.* reported a case study in order to determine the successful treatment of warts via local vitamin D activation in a patient with a kidney transplant. Eventually, they observed that active vitamin D had a significant effect on the treatment [15]. Furthermore, El-Taweel's recently published paper states that vitamin D has been successfully used in locally injectable forms [14].

According to the results of our study, the mean serum level of vitamin D in both case and control groups was 23, and about 50% of the subjects in both groups had insufficient levels. This deficiency of vitamin D and the development of warts show the importance of this issue.

In this regard, the results of a study by Liu *et al.* showed that vitamin D regulates some endogenous antimicrobial peptides in immune cells. This action leads to the potential role of vitamin D in regulating immune responses to various infectious diseases [16]. Additionally, Monto *et al.* in their study mentioned the hypothesis. They showed that the normal level of vitamin D in the host body contributed to the regulatory functions of the immune system against developing viral respiratory infections. These functions work through suppression of excessive responses to cytokines and increase the purification of various microbial species from the body [17]. These studies demonstrate the importance of vitamin D in preventing warts and are aligned with our study.

In addition, the number of warts in the case group was determined at two levels, which were lower than 5% with 71.4% and higher than 5% with 28. In 2013, one study in the United States reported a prevalence of warts among person with age from birth to 17. The highest one was 6.8% in children aged 9 to 11 [6]. Moreover, two population-based studies showed that the prevalence of wart was 84.1% and 12.9% in the United States and Russia, respectively. Other studies reported the prevalence of 12% in children aged 6-4 years in the United Kingdom and 24% in adolescents aged 18-16 in Australia [5]. These results are slightly different from our study results due to differences in samples and populations.

According to the results of our study, approximately 70% of the subjects had moderate levels of sun exposure. Paskit *et al.*, from 1974 to 1977, showed that exposure to sunlight has a significant effect on the level of vitamin D. They also found that in the winter, levels of this vitamin were more dependent on receiving the light than nutrition [13]. Cannele *et al.* concluded in their study that vitamin D deficiency could cause flu and respiratory infections in the winter due to lack of ultraviolet light [18]. Being consistent with our results, the results of these studies demonstrate the importance of exposure to the sun to provide adequate levels of vitamin D.

In our study, the amount of using sunscreen in both case and control groups was 21.4% and 39.3%, respectively. Russell *et al.* showed a correlation between vitamin D levels and skin moisture. According to this correlation, low level of vitamin D can decrease the average amount of skin moisture. Topical use of cholecalciferol improves skin moisture and improves the clinical grading of dry skin [19].

In addition, the rate of involvement in different

parts of the body was examined. Hands had the highest percentage. This indicates that some organs are more exposed to sunlight. Therefore, they have higher risk of developing warts, and it is better to use sunscreen for these places.

The serum levels of vitamin D in the groups with and without wart were compared to each other, which were not statistically significant in both genders. This issue suggests that the examination and follow-up of warts should be noticed in both genders with the same importance. Elaine *et al.* conducted a study in different age and sex groups. They showed that genital wart reductions were associated with the use of human papillomavirus vaccine. Furthermore, the prevalence of warts decreased over the years, and this drop occurred in females with different ages and in males aged 20 to 24 years old [20].

Moreover, the comparison of vitamin D in both groups was made based on age, and no significant relationship was found between them in both groups. This result suggests that warts may occur at any age. As humans grow up, more precise criteria are needed to determine the exact causes of warts. Raghukumar *et al.* conducted a study aiming at assessing the safety and efficacy of intracellular vitamin D in the treatment of warts. It is concluded that intravenous vitamin D is effective in the treatment of warts; however, aging can reduce the effect [2]. Contrary to our results, this study showed that aging was clearly an obstacle to the recovery of warts with vitamin D.

CONCLUSION

Consequently, despite the positive effect of vitamin D analogues on the treatment of warts, according to our results, there is no significant relationship between serum vitamin D levels and warts. This relationship is not statistically significant at the level of age and sex variables, and there is no difference in age and gender between the case and control groups.

Further studies in this field may also consider BMI to match the case and control groups to each other. It should also be noted that choosing control group from a larger population with various diets, in contrary to this research's second age group of controls, may lead to more realistic results. Both of these proposals were the limitations of this research, which can be resolved in future studies.

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