

Assessment of Serum 25- Hydroxycholecalciferol Level in Patients with Recalcitrant Palmoplantar Warts

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Abstract

Background: Viral warts are benign growths that result from an infection of epidermal or mucosal cells with the human papillomavirus. Epidemiological studies have demonstrated a link between vitamin D deficiency and increased rates of infectious diseases and their resistance to treatment. Such a link between vitamin D level and wart recalcitrance is yet to be revealed. To the best of our knowledge, there were no previous studies conducted to reveal the possible relationship between vitamin D and recalcitrant warts. **Aim:** To assess serum vitamin D levels in patients with recalcitrant palmoplantar warts. **Subjects and Methods:** A cross-sectional study conducted on two groups. The first group included 35 patients with recalcitrant palmoplantar warts, the second group included 35 healthy volunteers matched for age, sex. Serum vitamin D level was measured. **Results:** A total of 78 participants were enrolled in this study, including 30 males and 48 females, distributed among the 3 groups. There was no statistically significant difference in serum vitamin D levels between the studied groups. Its mean level in the recalcitrant group was 26.03 ng/ml, while in the responsive group it was 29.36 ng/ml and reached 25.15ng/ml in the control group. **Conclusion:** low serum vitamin D levels do not seem to increase the susceptibility of warts to get resistant to treatment.

Keywords: Warts, Recalcitrance, Serum vitamin D, Human Papilloma Virus

Introduction

Cutaneous viral warts represent a major economic and public health problem according to the 2010 Global Burden of Disease study⁽¹⁾. The greatest incidence of affection occurs between 12 and 16 years of age, with the peak incidence at age 13 years in females and age 14.5 years in males⁽²⁾. Although it is a benign condition, it has insightful appearance, tends to koebnerize, and can be transmitted to

others. This makes adequate and timely treatment pivotal⁽³⁾. Despite evidence-based guidelines for management of warts, a significant proportion of common warts fail to resolve, these are referred to as “recalcitrant warts”. There is no consensus on a single definition of recalcitrant warts⁽⁴⁾. However, it has been suggested to define them as those that fail to respond to one or more treatment modalities⁽⁵⁾. Up to one-third of non-genital warts are expected to be recalcitrant, es-

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pecially the plantar, periungual and sub-ungual types⁽⁵⁾. No single satisfying explanation is there for why some warts get resistant to treatment while others respond properly. Many modalities are available for the treatment of recalcitrant multiple common warts, but none of them offer a guarantee of cure⁽⁶⁾. During the past decade, better understanding of vitamin D actions, that exceed its bone formation, has emerged, and there has been much interest in its potential antimicrobial role against several bacterial and viral infections⁽⁷⁾. Epidemiological studies have demonstrated a link between vitamin D deficiency and increased rates of infectious diseases⁽⁷⁻⁹⁾. It is well established that topical vitamin D₃ analogues have some biological actions in epidermal cells, such as regulation of cell proliferation and differentiation and modulation of their cytokine production; increasing the expression of involucrin, transglutaminase, loricrin, and filaggrin⁽¹⁰⁾. To the best of our knowledge, serum vitamin D levels have not been assessed before as a possible association with recalcitrant or recurrent warts. This study plans to find out whether there is such an association thus giving us a better understanding of such intractable infections and opening the way for better future management approaches.

Subjects and Methods

A cross-sectional comparative study was carried out at the Dermatology Outpatient Clinic and clinical pathology department, Suez Canal University Teaching Hospitals, Ismailia, Egypt. An Approval was acquired from the Ethics committee. Written informed consent was taken from each participant. Patients were selected using consecutive non-probability sampling technique. The study included 3 groups: Group 1: included 19 patients with recalcitrant warts who failed to show any response to

one or more standard treatment modalities (six sessions of cryotherapy with an average of one to two weeks apart)⁵, Group 2: included 28 patients with responsive warts who showed response to less than 6 sessions of cryotherapy, Group 3: Included 31 patients, age and sex matched individuals as a control group. We excluded patients on vitamin D supplementation or using cod-liver oil, which contains a high concentration of vitamin D or receiving phototherapy, female patients who are pregnant, lactating or using oral contraceptives. Young adults with juvenile idiopathic arthritis or adults with rheumatoid arthritis or any other autoimmune or inflammatory diseases. Patients known to have malabsorption, short bowel, or cholestatic liver disease. Use of anticonvulsants, rifampicin, cholestyramine, highly active antiretroviral treatment (HAART), or glucocorticoids. All patients included in the study were subjected to full history taking, general and dermatological examination.

Assessment of serum 25- hydroxycholecalciferol level

Serum 25- hydroxycholecalciferol level was measured by vitamin D Enzyme-linked immunosorbent assay (ELISA) kit manufactured by Orgentec Diagnostika GmbH, Germany.

Assay procedure: The determination of serum 25-OH vitamin D level was based on a competitive ELISA with the following steps: The released 25-OH vitamin D sample was transferred to reaction wells of the microtiter plate. 25-OH vitamin D in the sample competed with the 25-OH vitamin D tracer reagent for binding to the 25-OH vitamin D antibodies coated onto the microwells. Complexes were formed between antibody and 25-OH vitamin D or antibody and 25-OH vitamin D tracer. After incubation, a first washing step removed

unbound and unspecifically bound molecules. Subsequently added enzyme conjugate bound to the immobilized tracer-antibody complexes. After incubation, a second washing step removed unbound enzyme conjugate. Addition of enzyme substrate solution resulted in blue color development during incubation. Addition of an acid stopped the reaction generating a yellow end-product. The intensity of the yellow color correlated inversely with the concentration of vitamin D in the sample and could be measured photometrically at 450nm.

Statistical analysis

Data was collected and coded then entered into spreadsheets using Microsoft Excel 2010 for Windows, of the Microsoft Office bundle; 2010 of Microsoft Corporation, United States. Data was analyzed using IBM Statistical Package for Social Sciences software (SPSS), 21st edition, IBM, United States. Normally distributed continuous data was expressed as mean \pm standard deviation. Categorical data was displayed as frequency and percentage. Data was presented as tables and graphs. Kruskal Wallis test was used to compare

between more than 2 not-normally distributed variables. Chi-squared test was used to compare between the qualitative data expressed as number and percentage, wherever compatible. Correlation (Spearman test) was used to identify relations between data and results were considered statistically significant at a p-value <0.05 .

Results

A total of 78 participants were enrolled in this study, including 30 males and 48 females, distributed among 3 groups: a recalcitrant to treatment group, a responsive to treatment group and a control one. Patients in both recalcitrant and responsive groups were selected from those who have palmer, planter or both palmer and planter warts. Their ages ranged between 19 and 62 years with mean of 33 ± 11.37 . Most of the patients were of skin type IV. The most frequent occurrence per control group is skin type III. There was no statistically significant difference in serum vitamin D levels between study groups (p value = 0.471) with the mean level was 26.03 ± 14.25 (Table 1).

Table 1. Serum vitamin D levels and daily sunlight exposure among resistant, responsive and control groups				
Group	Recalcitrant Group	Responsive Group	Control Group	p-value
Serum Vitamin D (ng/ml)	26.03 ± 14.25	29.36 ± 12.39	25.15 ± 14.06	0.471*
Daily sunlight exposure (hrs.)	3.25 ± 3.37	2.61 ± 2.87	2.13 ± 1.62	0.824*
Data are presented as mean \pm SD, P= Kruskal Wallis test; *=Statistically significant				

Patients were divided in groups according to serum vitamin D level (Table 2). There was no statistically significant difference between study groups regarding daily exposure to sunlight as shown in (Table 1). In addition, as would be expected, serum vitamin D levels went parallel with average daily sun exposure hours (figure 1). There were significant positive correla-

tions between serum vitamin D level and daily exposure to sun (p= 0.041) (Table 3).

Discussion

Although what we have so far of the aforementioned facts, and to the best of our knowledge, serum vitamin D level in patients with recalcitrant

warts has not yet been estimated as a possible association with such stubborn infection. Such association if proved could open the door wide to clearer understanding of the mechanism by which such infection becomes unresponsive to treatment in some patients, and thus paving the way towards more effective management Shim and his colleagues found increased prevalence of cervi-

covaginal warts among those with low levels of vitamin D⁽¹¹⁾. That contradicts Garcia's study although they studied the same type of warts⁽¹²⁾. Hence, further studies were to be held. It was suggested that there may be an etiological role of vitamin D deficiency and common wart infection following finding of increased prevalence of its deficiency among those affected with warts^(13,14).

Table 2. Vitamin D sufficiency level distribution among resistant, responsive and control groups					
Group	Resistant n (%)	Responsive n (%)	Control n (%)	Total n (%)	p-value
Deficient	1 (5.3)	2 (7.1)	6 (19.4)	9 (11.5)	0.242*
Insufficient	7 (36.8)	5 (17.9)	6 (19.4)	18 (23.1)	
Sufficient	11 (57.9)	21 (75)	19 (61.3)	51 (65.4)	
Total	19 (100)	28 (100)	31 (100)	78 (100)	
Chi-square test; *=Statistically significant at $p \leq 0.05$. serum vitamin D: sufficient (>20-150 ng/ml), insufficient (12-20 ng/ml), deficient (<12 ng/ml)					

We found that serum vitamin D levels in patients with recalcitrant warts were generally lower than that in the control group, and its level among those responsive to treatment are in general higher than that in the control ones, which may indicate higher response rates going with higher serum levels of vitamin D and vice versa. However, there was no bio-statistical significance of such variation. In other words, wart recalcitrance to treatment has no significant association with patients' serum 25-hydroxycholecalciferol level. This can be explained by Autier and his col-

leagues⁽¹⁵⁾ who thought that the strong association between low serum vitamin D levels and different inflammatory and infectious diseases was just a marker of generally ill health. The inflammatory process by itself involved in disease occurrence and clinical course would be the one accused for reducing serum vitamin D levels. This would explain its low-level association with a wide array of different unrelated diseases. In other words, it may be the result and not the cause of physiological disturbances accompanying some diseases⁽¹⁵⁾.

Table 3. Correlation between vitamin D and study variables in resistant, responsive and control groups.		
Variable 1	ρ	p-value
Average daily sunlight exposure (hrs.)	0.234	0.041*
Number of lesions	0.104	0.496*
Disease duration (months)	0.217	0.152*
Spearman correlation test; ρ = Spearman's correlation coefficient. *= Statistically significant at $p \leq 0.05$		

Study Limitations

A limitation to our study is that it is an observational one, and because serum vitamin D level is influenced by a wide array of factors e.g. aging, latitude, adiposity, physical activity, UV light exposure, smoking, and diet⁽¹⁶⁾ that are hard to be precisely controlled, resulting in an inevitable confounding bias to our data. Most of our knowledge of vitamin D and its association with different diseases are based mainly on observational studies as was ours. Although intervention studies will give much yield, observational studies still carry on their shoulders a big task of unveiling vitamin D secrets as randomized controlled trials still have a lot of limitations that limit their validity⁽¹⁷⁾. Furthermore, serum vitamin D can be influenced by Genetic and epigenetic factors⁽¹⁸⁾. That further disturbs the homogenization of target population among different studies, thus, biasing their results. Another limitation was the relatively small study population which might hinder extrapolation of the results. Further studies on wider scales might reveal more reliable results. Further and deeper research is needed to enlighten this area of our knowledge, and to either prove it more or decline its significance. Such findings are still recent and need more studies with larger number of included participants to reach solid ground in such new prospective of individualized wart treatment approach. This may help more homogenization of selected patients with viral warts in upcoming studies after revealing such a possible confounding factor.

Conclusion

There was no statistically significant difference in serum vitamin D levels between the studied groups as low serum vitamin D levels do not seem to increase the susceptibility of warts to get resistant to treatment.

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