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Research Article

The Relationship between Serum Vitamin D Levels and Viral Warts: A Case-Control Study

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Abstract

Background: Vitamin D has a role in regulating both adaptive and innate immunity, playing a key part in antimicrobial responses. ***Objective***: To investigate the relation between serum vitamin D levels and the presence and clinical characteristics of patients with viral warts. ***Methods***: A case-control study was conducted at Tikrit Teaching Hospital and a private clinic in Baghdad, Iraq, from April 2023 to August 2024. One hundred patients with warts and 100 healthy controls were enrolled. Demographic data, sun exposure, wart type, wart duration, and family history of warts were collected. Levels of vitamin D were assessed for all participants and categorized as deficient, insufficient, or sufficient. ***Results***: Vitamin D status revealed that 65% of wart cases and 72.2% of controls had sufficient levels. A family history of warts was more common in the deficient (40%) and sufficient (35.4%) groups than in the insufficient vitamin D status. Wart characteristics showed that the deficient and insufficient groups had only primary lesions, while the sufficient group had 43.1% primary and 56.9% recurrent lesions. All individuals in the deficient group had warts for less than one year, contrasting with 85.0% in the insufficient group and 35.4% in the sufficient group. ***Conclusions***: Vitamin D levels were related to specific clinical features of viral warts, with an insignificant difference in vitamin D levels found between the case and the control groups.

Keywords: Case-control, Viral warts, Vitamin D.

العلاقة بين مستويات فيتامين د في الدم والثآليل الفيروسية: دراسة حالة – شاهد

الخلاصة

الخلفية :يلعب فيتامين دال دورًا في تنظيم المناعة التكيفية والفطرية، وله دور رئيسي في الاستجابة المضادة للميكروبات. **الهدف :**دراسة العلاقة بين مستويات فيتامين دال في الدم ووجود الثآليل الفيروسية والخصائص السريرية للمصابين بها. **الطرائق:** أجريت دراسة حالة–شاهد في مستشفى تكريت التعليمي وفي عيادة خاصة في بغداد، العراق، من أبريل 2023 إلى أغسطس 2024. تم إشراك مائة مريض مصاب بالثآليل ومائة شخص سليم كمجموعة ضابطة. جُمعت البيانات الديموغرافية، والمعلومات عن التعرض لأشعة الشمس، ونوع الثآليل، ومدة الإصابة، والتاريخ العائلي للإصابة بالثآليل، وتم قياس مستويات فيتامين دال لجميع المشاركين وتصنيفها إلى ناقصة، غير كافية، وكافية. **النتائج :**أظهرت حالة فيتامين دال أن 65% من حالات الثآليل و72.2% من الأصحاء لديهم مستويات كافية. كان التاريخ العائلي للإصابة بالثآليل أكثر شيوعًا في المجموعات ذات النقص (40%) والكافية (35.4%) للفيتامين دال مقارنةً بمجموعة المستوى غير الكافي. أظهرت خصائص الثآليل أن المجموعات ذات النقص والمستوى غير الكافي كانت لديها فقط ثآليل أولية، بينما المجموعة ذات المستوى الكافي كان لديها 43.1% ثآليل أولية و56.9% ثآليل متكررة. جميع الأفراد في مجموعة النقص كانت مدة إصابتهم أقل من سنة، بالمقارنة مع 85.0% في مجموعة المستوى غير الكافي و35.4% في مجموعة المستوى الكافي. **الاستنتاجات :**كانت مستويات فيتامين دال مرتبطة بخصائص سريرية معينة للثآليل الفيروسية، مع عدم وجود فرق ذي دلالة إحصائية في مستويات فيتامين دال بين المجموعتين (الحالات والشواهد).

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INTRODUCTION

Warts are common skin lesions characterized by solid, skin-colored or gray-brown bumps with a rough texture and a thickness of a few millimeters. They can appear as single or multiple lesions, and when they merge, they can form plaques or regular shapes on the rough surface. Some types of warts can cover larger areas of the body and are more extensive than typical warts. Generally, they are painless [1]. Warts are the most common outcome of a human papillomavirus (HPV) infection. Human papillomavirus infects squamous epithelial cells, leading to DNA replication within these cells, which ultimately results in the development of warts [2]. Although no precise data exists on the incidence of non-genital warts, studies suggest they are more common in children and adolescents. A 2013 study in the U.S. found a prevalence of warts at 6.8% in children aged 9 to 11. Additionally, population-based studies reported varying wart prevalence rates: 84.1% in the U.S., 12.9% in Russia, 12% in children aged 4 to 6 in the U.K., and 24% in adolescents aged 16 to 18 in Australia [3]. In Iraq, viral skin warts were the most prevalent in Diyala province, affecting 39.1% of patients enrolled, with the young adult males being the most affected [4]. In another study carried out in the Sulaimani region of

Kurdistan, hand warts, affecting 31% of students, were the most common [5]. The prevalence of viral warts in Al-Sader city-Baghdad was found to be 15.71% [6]. Several immunotherapeutic agents, such as imiquimod, HPV vaccine, BCG vaccine, cimetidine, levamisole, systemic zinc, and interferon, have been used effectively to treat warts [7]. However, treatment success often depends on factors like the virus type, patient's immune status, lesion number, and duration of warts, particularly in adults and immunocompromised individuals [8]. Recent studies have highlighted vitamin D's role in regulating both adaptive and innate immunity, playing a key part in antimicrobial responses by initiating immune signaling pathways [9-11]. Vitamin D deficiency affects about 1 billion people globally [12]. Its derivatives have been used successfully in wart treatment, likely by regulating cytokine production and promoting epidermal cell differentiation [13]. A study reported that both intralesional and topical preparations of vitamin D are effective treatments for warts, with topical vitamin D formulations having faster, better efficacy, less risk of recurrence, and fewer side effects [14]. However, no research has yet established a definitive causal relationship between serum vitamin D levels and warts. Additionally, previous studies have shown conflicting results regarding whether patients with warts have reduced serum vitamin D levels [1,15-17]. Given the potential link between serum vitamin D deficiency and the occurrence of warts, this study was aimed at investigating the relation between serum vitamin D levels and the presence and clinical characteristics of viral warts.

METHODS

Study design and participants

This case-control study was conducted at Dermatology Consultancy at Tikrit Teaching Hospital in Salah Al-Din Governorate and at a private clinic in Baghdad, Iraq, between April 2023 and August 2024. The inclusion criteria specified that the patient group comprised individuals who had been clinically diagnosed with warts by a senior dermatologist. In contrast, the control group included healthy individuals with no history of warts or other skin disorders. Exclusion criteria included individuals with chronic illnesses, such as diabetes, autoimmune diseases, or malignant diseases, which may affect vitamin D levels. Those currently taking vitamin D supplements or medications influencing vitamin D metabolism were also excluded. Pregnant or breastfeeding women were not eligible, nor were individuals with other dermatological conditions, such as psoriasis or eczema, aside from warts. Additionally, participants with known immune system disorders or those unable or unwilling to comply with the study protocols were excluded. A total of 215 participants were recruited to participate using a convenience sampling technique. Fifteen participants were excluded for various reasons. The remaining 200 participants were divided into two groups: 100 patients diagnosed with warts (patient group) and 100 healthy individuals (control group). All participants were matched based on age and gender to minimize confounding factors.

Data collection

Data were collected through a structured data collection form. It included questions on variables such as age, sex, and sun exposure, which was classified as adequate if the subject had at least 10 minutes of exposure per day on more than 3 days per week; otherwise, it was classified as inadequate [18]; type of wart, whether primary or recurrent; duration of the wart in years; and family history of warts. Additionally, patients and control groups were asked to undergo serum 25 hydroxy vitamin D testing, which was performed in the private laboratory using the ELISA technique (Shanghai Yehua Biological Technology, China). For this test, 5.0 ml of a fasting blood sample was drawn from each participant and centrifuged at 3000 rpm for 20 minutes to get serum. Serum vitamin D levels were classified into three categories: deficient (<20 ng/mL), insufficient (20-30 ng/mL), and sufficient (>30 ng/mL) [19].

Ethical approval

Ethical approval for the research was obtained from the Tikrit Teaching Hospital Scientific Committee. All procedures involving human participants performed in the present study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments. Verbal consent was obtained from all patients before starting data collection and after explaining the details of the study and assuring confidentiality.

Statistical analysis

R software packages were used for data processing, visualization, and statistical analysis ("R version 4.2.2, R Foundation for Statistical Computing, Vienna, Austria"). Continuous variables were expressed as means ± standard deviations (SD). Categorical variables were expressed as frequency and percentages. The Welch's t-test and one-way analysis of variance (ANOVA) were performed to test the differences in means. The difference between categorical variables was investigated using either the χ^2 test with yates' correction or Fisher's exact test, depending on the context. A *p*-value less than 0.05 was considered statistically significant.

RESULTS

In this case-control study, Table 1 shows that the mean age was slightly higher in the wart cases at 22.4 ± 18.3 years compared to 19.1 ± 11.4 years in the control group, though this difference was not

statistically significant (p= 0.11). The distribution of sex was also comparable, with 60% males in the wart group versus 47% in the control group (p= 0.06).

Table 1: Description of study parameters both in cases and control groups (n= 100 in each group)

Characteristic	Wart Cases,	Healthy Control,	*p*-value
Age (year)	22.4±18.3	19.1±11.4	0.11[a]
Sex			
Male	60(60)	54(47)	0.06[b]
Female	40(40)	61(53)	
Family history of wart (Yes)	30(30)	27(23.5)	0.3[b]
Sun exposure			
Adequate	61(61)	83(72.2)	0.082[b]
Inadequate	39(39)	32(27.8)	
Lesion type			
Primary	63(63)	-----	----
Recurrent	37(37)	-----	
Duration of wart			
Less than 1 year	55(55)	----	----
1-2 year	25(25)	----	
More than 2 years	20(20)	----	
Vitamin D status			
Sufficient (>30 ng/mL)	65(65)	83(72.2)	
Insufficient (20-30 ng/mL)	20(20)	23(20)	0.2[b]
Deficient (<20 ng/mL)	15(15)	9(7.8)	

Values were expressed as frequency, percentage, and mean±SD. [a] Welch two sample t-test, [b] Chi-squared test.

A family history of warts was present in 30% of the wart cases and 23.5% of the controls (p= 0.3), indicating no significant familial predisposition in either group. Sun exposure habits varied, with 61% of cases reporting adequate sun exposure compared to 72.2% in the control group (p= 0.082). Among wart cases, 63% had primary lesions, while 37% experienced recurrent lesions. The duration of warts also differed, with 55% having a duration of less than one year, 25% between one and two years, and 20% over two years. Vitamin D status showed that 65% of wart cases and 72.2% of controls had sufficient levels, 20% of each group had insufficient levels, and 15% of wart cases versus 7.8% of controls had deficient levels, with no significant difference observed (p= 0.2). Table 2 examined the demographics and wart characteristics in patients stratified by vitamin D status into deficient (<20 ng/mL), insufficient (20-30 ng/mL), and sufficient (>30 ng/mL) groups. The mean age increased significantly across these groups, with the deficient group averaging 6.4 ± 2.3 years, the insufficient group at 15.6 ± 2.4 years, and the sufficient group notably older at 28.3 ± 20.1 years (p< 0.001). Sex distribution was fairly balanced across groups, with no significant difference observed (p= 0.2).

Table 2: Demographics and wart characteristics stratified by vitamin D status

Characteristic	Deficient <20 ng/mL (n= 15)	Insufficient 20-30 ng/mL (n= 20)	Sufficient >30 ng/mL (n= 65)	*p*-value
Age (year)	6.4±2.3	15.6±2.4	28.3±20.1	<0.001[a]
Sex				
Male	8(53.3)	9(45)	43(66.2)	0.2[b]
Female	7(46.7)	11(55)	22(33.8)	
Family history of wart (Yes)	6(40)	1(5)	23(35.4)	0.014[c]
Sun exposure				
Adequate	11(73.3)	8(40)	42(64.6)	0.081[c]
Inadequate	4(26.7)	12(60)	23(35.4)	
Lesion type				
Primary	15(100)	20(100)	28(43.1)	<0.001[c]
Recurrent	0(0.0)	0(0.0)	37(56.9)	
Duration of wart				
Less than 1 year	15(100)	17(85)	23(35.4)	
1-2 year	0(0.0)	0(0.0)	25(38.5)	<0.001[c]
More than 2 years	0(0.0)	3(15)	17(26.2)	

Values were expressed as frequency, percentage, and mean±SD. [a] Welch two sample t-test, [b] Chi-squared test, [c] Fisher's exact test.

A family history of warts was more common in the deficient (40%) and sufficient (35.4%) groups than in the insufficient group (5.0%), yielding a statistically significant difference (p= 0.014). Sun exposure varied, with 73.3% of the deficient group reporting adequate exposure, while this was lower in the insufficient group (40.0%) and moderate in the sufficient group (64.6%), though this difference did not reach statistical significance (p= 0.081). Wart lesion type and duration showed clear relations with vitamin D status. All individuals in the deficient and insufficient groups had primary lesions, while the sufficient group had a mix of primary (43.1%) and recurrent (56.9%) lesions (p< 0.001). The duration of warts also varied significantly; 100% of the deficient group had warts for less than one year, compared to 85.0% in the insufficient group and 35.4% in the sufficient group, with longer durations more prevalent in the sufficient group (p< 0.001).

DISCUSSION

Vitamin D deficiency is commonly observed in high-risk patient populations, but its prevalence among otherwise healthy adults, particularly those with viral warts, is less well-defined. Current guidelines recommend screening only individuals with known risk factors for vitamin D deficiency, which excludes a significant portion of the population, including those who may be susceptible to conditions like viral warts [20]. An insignificant difference was found in serum levels of vitamin D in patients with viral warts compared to controls (p= 0.2), despite lower levels of vitamin D that were observed among wart cases. Supporting these findings, a study by Goodarzi *et al*. [1] in Iran similarly concluded that no significant relationship exists between serum vitamin D levels and the presence of warts, with no statistical significance based on age or sex. Mertoğlu *et al*. [21] also reported no difference in vitamin D levels between individuals who tested positive and negative for HPV infection. Shalaby *et al*. [13] observed that 35% of patients had sufficient levels of vitamin D, 40% had insufficient levels, and 25% were deficient, with no significant difference in vitamin D deficiency status between patients and healthy controls. Cao *et al*. [17] conducted a study in China and found that the serum vitamin D levels in children with warts did not significantly differ from those in children with other common skin conditions in the control group. In addition, Khalili *et al*. [22] conducted a systematic review of seven studies involving 11,168 participants and found no conclusive evidence linking serum vitamin D levels with cervicovaginal human papillomavirus infection. In contrast, a study conducted by Mongy *et al*. [15] in Egypt reported a significant reduction in serum 25-hydroxy vitamin D levels in patients with viral warts compared to controls (p= 0.001). Kanwal *et al*. [23] conducted a study in Pakistan, which revealed a statistically significant reduction in serum 25-hydroxy vitamin D levels (p= 0.004) among patients with viral warts. The same observation was found by Öztekin *et al*. [24] study in Türkiye. Differences from the current study's findings might be attributed to factors such as limited exposure to sunlight due to local dress customs, cultural practices, low levels of outdoor activity, air pollution, and insufficient dietary intake of vitamin D in addition to the selection of the control group in each study [25]. Due to the widespread prevalence of vitamin D deficiency, individuals who are considered "healthy" may still have low vitamin D levels. For instance, Tamer *et al*. have found that the average serum vitamin D levels in control groups are also below 20 ng/mL, which suggests a deficiency [26]. This raises questions about the validity of using a "healthy population" as a control group, as they may not be truly sufficient vitamin D. While this study did not find a statistically significant difference in serum vitamin D levels between patients with warts and the control group, it may not completely rule out a potentially significant relationship between serum vitamin D levels and the presence of warts. The role of vitamin D in wart treatment remains incompletely understood, but it is anticipated to exert immunoregulatory effects by influencing epidermal cell growth and cytokine production [27]. Additionally, it is proposed that vitamin D regulates the production of antimicrobial peptides (AMPs) like cathelicidin LL-37, α-defensin, and β-defensin in epidermal cells, which could otherwise be limited by vitamin D deficiency, raising susceptibility to viral infections. Additionally, vitamin D enhances physical barriers—such as the respiratory, skin, and genitourinary tracts—against bacterial and viral pathogens by upregulating proteins involved in tight, gap, and adherence junctions [28]. This study also reported a direct relation between the age of patients with warts and vitamin D levels, as these levels increased with the progression in the age of the patients. Chalcraft *et al*. reported that serum vitamin D levels in younger and older adults increased significantly in response to outdoor sunlight exposure. Even though aging may inhibit cutaneous synthesis, sunlight exposure is still an important source of vitamin D [29]. A significant difference in vitamin D levels among viral warts patients was recorded in this study in regard to the family history of viral warts. Shalaby *et al*. reported that male/female gender did not significantly alter the serum levels of vitamin D (p= 0.112); on the other hand, they reported that family history of warts did not significantly affect serum vitamin D levels [13]. A significant difference in vitamin D levels among viral wart patients was recorded in this study in regard to the type of warts. Also, a significant difference in vitamin D levels among these patients was recorded in this study in regard to the duration of wart lesions. Shalaby *et al*. reported that neither the duration nor the number of warts was significantly correlated with serum vitamin D levels [13]. This study is limited by the number of participants that was restricted to one center and one private clinic; therefore, generalizability of the findings is inadequate.

Conclusion

Vitamin D levels were associated with specific clinical features of viral warts, although an insignificant difference in serum vitamin D levels was found between the case and the control groups. Also, vitamin D deficiency may affect both susceptibility to and progression of viral warts. Future research is required to investigate the effects of vitamin D supplementation on wart recurrence and healing and to examine how vitamin D may modulate immune responses against viral infections.

Conflict of interests

The author declares no conflict of interest.

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Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

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