



Lower Levels of Vitamin B12 Among Patients with Viral Warts Compared with Control Subjects: A Retrospective Study

Viral Siğili Olan Hastalarda Kontrol Grubuna Kıyasla Daha Düşük Vitamin B12 Seviyeleri:
Retrospektif Bir Çalışma

Gökşen ERTUĞRUL

 0000-0002-5167-4780

Habibullah AKTAŞ

 0000-0001-9239-1659

Department of Dermatology, Karabük
University Faculty of Medicine,
Karabük, Turkey

ABSTRACT

Aim: Viral wart is a common benign infectious dermatosis. It remains unclear why not everyone exposed to HPV develops warts. It is known that vitamin and mineral deficiencies can affect impair natural and acquired immunity and lead to a tendency to get more infections. This study aimed to determine the potential utility of evaluating vitamin B12, folic acid, and ferritin levels in patients with viral warts.

Material and Methods: In this retrospective study, 70 patients who were diagnosed with viral warts in the dermatology outpatient clinic of Karabük University Training and Research Hospital between January 2018 and December 2019 were included. 70 healthy individuals matched with viral wart patients in terms of gender and age were included as the control group. Serum vitamin B12, folic acid, and ferritin levels of both groups were detected and compared from the hospital automation system.

Results: Vitamin B12 level was detected statistically significantly lower in the viral wart group as compared to the control group ($p=0.046$). Although serum ferritin levels were found lower in the viral wart group, this difference was not statistically significant ($p=0.677$). No statistically significant difference was detected between the viral wart and control groups in terms of folate levels ($p=0.879$).

Conclusion: In patients with viral warts with low serum vitamin B12 levels, replacement therapy could be considered. Also, the evaluation of serum ferritin level, an iron storage parameter in the body, is important in patients with viral warts infection.

Keywords: Wart; vitamin B12; folic acid; ferritin; viral warts.

ÖZ

Amaç: Viral siğil, yaygın olarak görülen benign enfeksiyöz bir dermatozdur. HPV'ye maruz kalan herkesin neden siğil geliştirmedeği belirsizliğini korumaktadır. Vitamin ve mineral eksikliklerinin doğal ve kazanılmış bağışıklığı zayıflatabileceği ve daha fazla enfeksiyon kapma eğilimine yol açabileceği bilinmektedir. Bu çalışma, viral siğili olan hastalarda vitamin B12, folik asit ve ferritin düzeylerinin değerlendirilmesinin potansiyel faydasını değerlendirmeyi amaçlamıştır.

Gereç ve Yöntemler: Bu geriye dönük çalışmaya, Ocak 2018 ve Aralık 2019 tarihleri arasında Karabük Üniversitesi Eğitim ve Araştırma Hastanesi dermatoloji polikliniğinde viral siğil tanısı almış olan 70 hasta dahil edildi. Kontrol grubu olarak cinsiyet ve yaş açısından viral siğil hastaları ile uyumlu olan 70 sağlıklı birey dahil edildi. Hastane otomasyon sisteminden her iki grubun serum vitamin B12, folik asit ve ferritin düzeyleri tespit edildi ve karşılaştırıldı.

Bulgular: Serum vitamin B12 düzeyi, viral siğili olan hasta grubunda kontrol grubuna göre istatistiksel olarak anlamlı derecede daha düşük tespit edildi ($p=0,046$). Serum ferritin düzeyleri viral siğil grubunda daha düşük bulunmakla birlikte, bu fark istatistiksel olarak anlamlı değildi ($p=0,677$). Folat seviyeleri açısından, viral siğil ve kontrol grupları arasında istatistiksel olarak anlamlı bir farklılık tespit edilmemiştir ($p=0,879$).

Sonuç: Serum vitamin B12 düzeyi düşük viral siğili olan hastalarda vitamin B12 replasman tedavisi düşünülebilir. Ayrıca viral siğil enfeksiyonu olan hastalarda vücutta demir depolama parametresi olan serum ferritin düzeyinin değerlendirilmesi önemlidir.

Anahtar kelimeler: Siğil; B12 vitamini; folik asit; ferritin; viral siğil.

Corresponding Author

Sorumlu Yazar

Gökşen ERTUĞRUL

goksenycl@hotmail.com

Received / Geliş Tarihi : 05.01.2022

Accepted / Kabul Tarihi : 03.04.2022

Available Online /

Çevrimiçi Yayın Tarihi : 17.04.2022

INTRODUCTION

Viral wart is benign infectious dermatosis manifested mainly by hyperkeratotic papules on the skin and mucous membranes. The causative human papilloma virus (HPV) often enters the body through areas in the skin where the epidermis is damaged and forms viral wart lesions. Complete recovery is expected in months or years based on the age and immune status of the patient, subtype of the causative HPV, and the region where the lesion develops (1). Viral warts are mostly cosmetic disorders, but they can sometimes be painful or cause secondary bacterial infection. Viral warts can also negatively impact the quality of life of patients (2). The treatment of warts generally includes local destructive methods and, local or systemic immunotherapy (3).

Vitamin B12 plays an important role in lipid, carbohydrate, and protein metabolism in the body (4). Inadequate intake or malabsorption of vitamin B12 could lead to vitamin B12 deficiency. This deficiency can cause multi-organic and systemic dysfunctions, especially in hematological, neurological, and neuropsychiatric systems (5). Changes in cellular metabolism affect the functions of cells involved in immunity (6). Vitamin B12 facilitates the production of T-cells, such as cytotoxic T-cells (7). In the case of vitamin B12 deficiency, B cell diversity and natural killer (NK) cell activity decrease, and antibody response is also impaired (7). Vitamin B12 has immunomodulatory effects, especially on cellular immunity. Vitamin B12 replacement decreases the CD4+/CD8+ ratio, and increases NK cell activity, in cases with vitamin B12 deficiency (8).

Iron is a mineral that has many biological functions in the body, such as oxygen transport to cells, destruction of pathogens, regulation of cytokine production and action, and IFN γ production (7,9,10). Iron is important in the differentiation and proliferation of T cells and helps to regulate the ratio of T helper cells and cytotoxic T cells (7). Iron homeostasis is tightly regulated in healthy individuals, as iron is required in the immune response of T-lymphocytes and antimicrobial mechanisms of macrophages (11,12). Serum ferritin level is an accessible and important parameter to evaluate the body's iron storage in the absence of systemic infection and inflammation (10).

Folate (vitamin B9) is involved in DNA synthesis and methylation reactions (13,14). Folate maintains or increases the cytotoxic activity of the NK cell, supports Th 1-mediated immune responses, and provides adequate antibody response to antigens (7). Cell culture studies have shown that folate deficiency favors a decrease in T-lymphocyte proliferation, an increase in CD4+ cells, a decrease in CD8+ cells, and consequently, a greater tendency to develop infections (15).

Antimicrobial substances, phagocytes, and NK cells in the serum constitute the body's natural immunity (7). Vitamins and trace elements in the body contribute to natural and acquired immunity; thus, vitamin and mineral deficiencies lead to weakness in immunity and a greater tendency to be infected (16). There is limited information in the literature about the relationship between viral, bacterial, and parasitic diseases of the skin and vitamin and mineral deficiencies.

In this study, we aimed to examine vitamin B12, folate, and ferritin levels in patients with viral warts.

MATERIAL AND METHODS

This retrospective study was conducted between January 2018 and December 2019 in the department of dermatology at a tertiary care hospital in Karabük. The study was performed in accordance with the principles of the Declaration of Helsinki and approved by our Institutional Ethical Committee (2021/609). A total of 70 patients with viral warts were enrolled in this study. All types of viral warts including common warts, verruca plana, genital warts, and plantar warts were included in the study. Cases that have a pregnancy, breastfeeding, malignancy, active infection, metabolic and endocrine disorders, liver disease, and also receive any replacement or drug therapy for vitamin B12, folic acid, and iron were excluded from the study. The control group consisted of 70 people that applied to the hospital for non-wart reasons, had similar age and gender characteristics with the patient group, and met the exclusion criteria. The age and gender of patients and control group were noted. The serum vitamin B12, folic acid, and ferritin levels of all patients were obtained from the hospital automation system. The min-max values of the laboratory parameters were considered normal for vitamin B12 (211-911 pg/mL), folic acid (5-17 ng/mL), and ferritin (22-322 ng/mL), respectively. Results below the minimum values for all parameters were considered deficient.

Statistical Analysis

Data analysis was performed using the Statistical Package for Social Sciences (SPSS) version 21 (SPSS software, Chicago, IL, USA), and p values less than 0.05 were considered statistically significant. The normality distributions of numerical variables were evaluated with the Kolmogorov-Smirnov test. In the comparison of paired groups, the Independent samples t-test was used for normally distributed variables while the Mann-Whitney U test was used for variables that were not normally distributed. Gender distribution was analyzed with the Pearson chi-square test. Normally distributed data were presented as mean \pm standard deviation while non-normally distributed data were presented as median (interquartile range) [minimum-maximum], and categorical data were presented with number and percentage.

RESULTS

The study group included 70 patients (52 females, 18 males), while the control group had 70 patients (46 females, 24 males). There was no significant difference in terms of gender between the groups ($p=0.268$). The mean age of the study group was 28.61 ± 11.73 (range, 14-69) years, while the mean age of the control group was 30.02 ± 11.23 (range, 15-56) years. There was no significant difference across the groups ($p=0.370$). The baseline demographics of the groups were shown in Table 1.

Serum vitamin B12 level was significantly lower in the viral wart group compared to the control group (302.54 ± 91.81 vs 331.49 ± 91.49 , $p=0.046$). While serum ferritin level was found to be lower in the viral wart group than in the control group (median 17 vs 20), the difference was not statistically significant ($p=0.677$). Also, there was no significant difference was found between folic acid levels of the wart group and the control group ($p=0.879$). Serum vitamin B12, folic acid, and ferritin levels of patient and control groups were given in Table 2.

Table 1. Baseline demographics of the participants

	Wart Group	Control Group	p
Age, mean±SD (min-max)	28.61±11.73 (14-69)	30.02±11.23 (15-56)	0.370
Gender, n (%)			
Male	18 (25.7%)	24 (34.3%)	0.268
Female	52 (74.3%)	46 (65.7%)	

Table 2. Serum vitamin B12, folic acid, and ferritin levels of the wart group and control group

	Wart Group	Control group	P
Vitamin B12	302.54±91.81	331.49±91.49	0.046
Ferritin	17 (25) [3-170]	20 (32.8) [4-200]	0.677
Folic acid	7.5 (3) [5-16]	7 (3) [4-19]	0.879

Descriptive statistics were presented as mean±standard deviation or median (interquartile range) [minimum-maximum]

DISCUSSION

The skin and mucous membranes are the first lines of defense, covering the outer and inner surfaces of the body against viruses, bacteria, and all pathogens. These physical barriers need maintenance for their integrity and optimal functioning. Micronutrients, including vitamin B12, iron, and folate, have vital roles in ensuring this maintenance (7).

In our study, serum vitamin B12 level was found to be significantly lower in the viral wart group. In their study including all wart types, Tamer et al. (17) found lower vitamin B12 levels in the patient group compared to healthy individuals, similar to the results of our study. Demir et al. (18) examined vitamin B12, ferritin, and some blood parameters in patients with genital warts. In their study, they did not find a relationship between genital warts and vitamin B12, but they found high levels of ferritin in patients with genital warts. The differences between our study and Demir et al. (18) study may be due to the wart types, age and gender distribution of the patients included in the study.

Another study again from Turkey investigated the presence of HPV in the cervical samples of women by a polymerase chain reaction and demonstrated that vitamin B12 and folate deficiencies were significantly higher in HPV (+) cases than in HPV (-) cases (19). In a Brazilian cohort study, it was shown that dietary intake of vitamin B12 is associated with a reduced risk of non-oncogenic HPV persistence in males (20). Similarly, in a study conducted on 201 female patients with persistent or intermittent cervical HPV infection, an inverse correlation was found between serum vitamin B12 values and HPV persistence (21). There is one more study suggesting lower vitamin B12 levels may be associated with high-grade cervical lesions that could be HPV-related (22). Although the viral subtypes are different in common warts and genital warts, all findings from these studies suggest that there may be an association between lower vitamin B12 levels and HPV infections or HPV virulence.

In this study, we found lower serum ferritin levels in the viral wart group, though not significant. Lactoferrin contributes to the body's natural immunity

and chelates iron and is widely found in body fluids and specific neutrophil granules of polymorphonuclear leukocytes (23). It can both prevent the entry of the virus into the cell and possess antiviral effects in the cell by being localized in the nuclear region of the cell. Antiviral effects of lactoferrin have been demonstrated against many viruses, including human papillomavirus (24). Therefore, the evaluation of serum ferritin level, an iron storage parameter in the body, is important in patients with viral warts infection.

Folate levels were not different between the groups in the presented study. Tamer et al. (17) also found similar folate values in the viral wart and control group in their retrospective case-control study. However, vitamin B12 deficiency leads to a metabolic block in the use of folate at the cellular level. Therefore the evaluation of folate levels in individuals with vitamin B12 deficiency may not be accurate. In the literature, it is recommended to reevaluate serum metabolites such as methylmalonic acid, homocysteine, and folate levels after vitamin B12 replacement therapy in individuals with vitamin B12 deficiency. Since serum homocysteine level increases in serum folate and vitamin B12 deficiencies, it has been reported that if the serum homocysteine level does not decrease after vitamin B12 replacement therapy, folate deficiency and replacement should be considered as well (25).

If the serum vitamin B12 level is <200 µg/mL, it is referred to as vitamin B12 deficiency. However, levels between 200-400 µg/mL are borderline, and reevaluation with methylmalonic acid and homocysteine levels is required to confirm the diagnosis (5). The methylmalonic acid level is considered more specific in the evaluation of vitamin B12 deficiency due to the high homocysteine level in the case of folate deficiency (26). When vitamin B12 deficiency is suspected from laboratory examination, further evaluation may be required along with the routine laboratory levels of the patients. Therefore, we think that if the vitamin B12 levels of patients with viral warts are at the borderline limit, they should be re-evaluated with these additional tests and the result should be confirmed.

This study has several limitations worth noting, firstly the retrospective design. It would be better if we could evaluate the response in viral warts after replacement in patients with low vitamin B12. Moreover, since serum folic acid level is affected by low vitamin B12 levels it would be logical to use rechecked folic acid levels after vitamin B12 replacement.

There are few studies currently available addressing the link between vitamin B12 levels and viral warts. In this study, we found significantly lower vitamin B12 levels in patients with viral warts compared to controls. Further studies are needed to confirm our results, and also to reveal the effect of vitamin B12 replacement in wart patients with low vitamin B12 levels.

CONCLUSION

Serum vitamin B12 levels should be checked in patients with viral warts, and replacement therapy could be considered in patients with low levels. Also, it is important to evaluate the serum ferritin level, an iron storage parameter in the body, in patients with viral warts infection.

Ethics Committee Approval: The study was approved by the Non-Interventional Clinical Research Ethics Committee of Karabük University (06.08.2021, 609).

Conflict of Interest: None declared by the authors.

Financial Disclosure: None declared by the authors.

Acknowledgments: None declared by the authors.

Author Contributions: Idea/Concept: GE, HA; Design: GE, HA; Data Collection/Processing: GE, HA; Analysis/Interpretation: GE, HA; Literature Review: GE, HA; Drafting/Writing: GE, HA; Critical Review: GE, HA.

REFERENCES

- Loo SK, Tang WY. Warts (non-genital). *BMJ Clin Evid* 2014;2014:1710.
- Salah E. Impact of multiple extragenital warts on quality of life in immune-competent Egyptian adults: a comparative cross-sectional study. *Clin Cosmet Investig Dermatol*. 2018;11:289-95.
- Abeck D, Tetsch L, Lüftl M, Biedermann T. Extragenital cutaneous warts - clinical presentation, diagnosis and treatment. *J Dtsch Dermatol Ges*. 2019;17(6):613-34.
- Romain M, Sviri S, Linton DM, Stav I, van Heerden PV. The role of vitamin B12 in the critically ill--a review. *Anaesth Intensive Care*. 2016;44(4):447-52.
- Oh R, Brown DL. Vitamin B12 deficiency. *Am Fam Physician*. 2003;67(5):979-86.
- Alwarawrah Y, Kiernan K, MacIver NJ. Changes in nutritional status impact immune cell metabolism and function. *Front Immunol*. 2018;9:1055.
- Gombart AF, Pierre A, Maggini S. A review of micronutrients and the immune system-working in harmony to reduce the risk of infection. *Nutrients*. 2020;12(1):236.
- Wintergerst ES, Maggini S, Hornig DH. Contribution of selected vitamins and trace elements to immune function. *Ann Nutr Metab*. 2007;51(4):301-23.
- Śliwińska A, Luty J, Aleksandrowicz-Wrona E, Małgorzewicz S. Iron status and dietary iron intake in vegetarians. *Adv Clin Exp Med*. 2018;27(10):1383-9.
- Lynch S, Pfeiffer CM, Georgieff MK, Brittenham G, Fairweather-Tait S, Hurrell RF, et al. Biomarkers of Nutrition for Development (BOND)-Iron Review. *J Nutr*. 2018;148(suppl_1):1001S-67S.
- Hassan TH, Badr MA, Karam NA, Zkaria M, El Saadany HF, Abdel Rahman DM, et al. Impact of iron deficiency anemia on the function of the immune system in children. *Medicine (Baltimore)*. 2016;95(47):e5395.
- Ganz T, Aronoff GR, Gaillard CAJM, Goodnough LT, Macdougall IC, Mayer G, et al. Iron administration, infection, and anemia management in CKD: untangling the effects of intravenous iron therapy on immunity and infection risk. *Kidney Med*. 2020;2(3):341-53.
- Ebara S. Nutritional role of folate. *Congenit Anom (Kyoto)*. 2017;57(5):138-41.
- Socha DS, DeSouza SI, Flagg A, Sekeres M, Rogers HJ. Severe megaloblastic anemia: Vitamin deficiency and other causes. *Cleve Clin J Med*. 2020;87(3):153-64.
- Partearroyo T, Úbeda N, Montero A, Achón M, Varela-Moreiras G. Vitamin B(12) and folic acid imbalance modifies NK cytotoxicity, lymphocytes B and lymphoproliferation in aged rats. *Nutrients*. 2013;5(12):4836-48.
- Calder PC, Carr AC, Gombart AF, Eggersdorfer M. Reply to "Comment on: optimal nutritional status for a well-functioning immune system is an important factor to protect against viral infections. *Nutrients* 2020,12,1181". *Nutrients*. 2020;12(8):2326.
- Tamer F, Yuksel ME, Karabag Y. Pre-treatment vitamin B12, folate, ferritin, and vitamin D serum levels in patients with warts: a retrospective study. *Croat Med J*. 2020;61(1):28-32.
- Demir A, Güder S. The effects of vitamin B12, vitamin D, ferritin level, neutrophil/monocyte ratio and some blood parameters on genital warts presence, the number of lesions, and recurrence rates. *Andrologia*. 2021;53(4):e14006.
- Yenigül NN, Yazıcı Yılmaz F, Ayhan I. Can serum vitamin B12 and folate levels predict HPV penetration in patients with ascus? *Nutr Cancer*. 2021;73(4):602-8.
- Lopes RDVC, Teixeira JA, Marchioni D, Villa LL, Giuliano AR, Luiza Baggio M, et al. Dietary intake of selected nutrients and persistence of HPV infection in men. *Int J Cancer*. 2017;141(4):757-65.
- Sedjo RL, Inserra P, Abrahamsen M, Harris RB, Roe DJ, Baldwin S, et al. Human papillomavirus persistence and nutrients involved in the methylation pathway among a cohort of young women. *Cancer Epidemiol Biomarkers Prev*. 2002;11(4):353-9.
- Silva NNT, Silva Santos AC, Carneiro CM, Lima AA. Association of serum folate and vitamin B12 with pre-neoplastic cervical lesions. *Clin Nutr ESPEN*. 2020;38:223-8.
- Redwan EM, Uversky VN, El-Fakharany EM, Al-Mehdar H. Potential lactoferrin activity against pathogenic viruses. *C R Biol*. 2014;337(10):581-95.
- Habib HM, Ibrahim S, Zaim A, Ibrahim WH. The role of iron in the pathogenesis of COVID-19 and possible treatment with lactoferrin and other iron chelators. *Biomed Pharmacother*. 2021;136:111228.
- Antony AC. Evidence for potential underestimation of clinical folate deficiency in resource-limited countries using blood tests. *Nutr Rev*. 2017;75(8):600-15.
- Shipton MJ, Thachil J. Vitamin B12 deficiency - A 21st century perspective. *Clin Med (Lond)*. 2015;15(2):145-50.