Exploring The Relationship Between Vitamins B12 and Folic Acid in Newly Diagnosed Cutaneous Wart Patients.

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Abstract:

Background: In the field of dermatology, it has been suggested that the combined supplementation of vitamins B12 and folic acid may improve the effectiveness of cutaneous wart treatments. These vitamins play crucial roles in supporting skin health and enhancing immune function, both of which are essential for the body's ability to fight off viral infections like those causing cutaneous warts. **Objectives of study:** To determine the potential utility of evaluating vitamin B12, folic acid in newly diagnosed patients with cutaneous warts. **Patients and methods:** The study included 88 individuals, 38 healthy controls without warts, and 50 patients newly diagnosed with cutaneous warts by a dermatologist. Among the patients, 23 were men and 27 were women, with ages ranging from 10 to 60 years. further subdivided into Group A (25 with plantar warts) and Group B (25 with flat warts), it is worth mentioning that each of the serum vitamin B12 and folic acid was determined by using an enzyme-linked immunosorbent assay. **Result:** The study found a significant decrease in serum folic acid {with mean ±SD (1.448±0.16) Nmol/L} and B12 levels {with mean ±SD (40,38±17.75) Pmol/L} in cutaneous wart patients with ($p \leq 0.05$), compared to serum folic acid (23.49±6.25) Nmol/L and serum vitamin B12 (281.20±115.3) Pmol/L in healthy individuals with ($p \leq 0.05$). non-significant differences were observed in vitamin levels between patients with plantar warts or flat warts with ($p \geq 0.05$). There was no significant correlation between vitamin B12 and folic acid in either healthy individuals or patients with cutaneous warts ($p \geq 0.05$). **Conclusion:** Research results suggest that folic acid and B12 deficiencies may be linked to the presence of cutaneous warts.
Keywords: Vitamin B12, Folic acid, and Wart.

Introduction:

Warts, also known as verrucae, are common benign skin growths caused by the human papillomavirus (HPV). They are non-cancerous and result from viral infections that stimulate rapid cell proliferation in the epidermis. warts can appear anywhere on the body and affect individuals of all ages (**Zhu, Qi et al. 2022**). The human papillomavirus often enters the body through areas in the skin where the epidermis is damaged and forms viral wart lesions **(Loo and Tang 2014**). The clinical manifestations depend on the HPV type involved, the anatomic location, and the immune status of the host (**Smith 2008**). Cutaneous and mucosal HPV types form two distinct groups that infect either skin or mucosa (**Bolognia, Jorizzo et al. 2012**). HPV can be passed through any infected skin-to-skin contact or by touching a surface that carries the virus. Warts often appear on hands and feet because the hands and feet frequently contain minor skin irritations where the virus can penetrate (**Kadhom, Ali et al. 2023**). The role of the immune system in wart persistence and resolution is well-documented. emerging evidence highlights the significance of micronutrient status, particularly vitamins B12 and folic acid, in modulating immune function and viral dynamics (**Singh, Chouhan et al. 2014**).

Both vitamins B12 and folic acid are involved in the methylation cycle and homocysteine metabolism, and their deficiencies often occur together. Deficiencies in either vitamin can result in hyperhomocysteinemia, a condition that has been linked to oxidative stress and endothelial dysfunction, both of which may exacerbate dermatological conditions **(Smith 2008**). Additionally, the combination of B12 and folic acid deficiencies can impair the body's ability to repair skin damage (**Diotallevi, Campanati et al. 2022**), leading to delayed wound healing and increased susceptibility to dermatological infections **(Zhu, Chen et al. 2023**).

Both vitamins are essential for T-cell proliferation and antibody production **(Mora, Iwata et al. 2008**). deficiencies correlate with reduced

cytotoxic T-cell activity, potentially exacerbating HPV persistence. Emerging studies suggest that adequate B12 and folic acid levels enhance the clearance of HPV lesions. (**Ferrari, Magni et al. 2023**)

Both folic acid and B12 work synergistically to support cellular processes and immune function (**Ertuğrul and Aktaş 2022**). The deficiencies in these vitamins can lead to anemia and reduced immune competence, creating an environment in which HPV can thrive. ensuring adequate intake of these vitamins through diet or supplementation can improve the body's ability to combat infections (**Mikkelsen and Apostolopoulos 2019**). Our aim of study was to investigate whether there is a significant correlation between the serum VB12 and folic acid levels and the occurrence of cutaneous warts in patients within the age range of 10-60 years. This will help determine if deficiencies in these vitamins are more prevalent in individuals with cutaneous warts, which could offer insight into underlying health conditions that may contribute to wart development, as well as to compare serum vitamin B12 and folic acid levels in patients with newly diagnosed cutaneous warts to those without warts

Patients and methods:

Study design, Date and Place of the work:

The study took an analytical cross-sectional statistical design, which was conducted from February to October 2024 at Baghdad Medical City Complex in Baghdad, Iraq.

Ethical Considerations:

Official approval was granted by the University of Baghdad/College of Medicine/Department of Clinical Chemistry. Additionally, official approval was obtained from the Iraqi Board for Medical Specializations and the Ministry of Health and Environment, as well as the oral consent of all patients included in the study.

Subjects and sampling procedure:

The study analyzed the data of 88 individuals,38 of whom were considered healthy individuals without cutaneous warts; the rest were 50 patients with cutaneous warts diagnosed by a specialized dermatologist; men with warts represent 23, while 27 were women. The following are the details of the groups in the study:

Groups:

Group 1: 38 healthy individuals, without cutaneous warts
Group 2: 50 patients newly diagnosed with cutaneous warts

Subgroups:
Group B: 25 patients with flat warts, both males and females.
Group A: 25 patients with plantar warts, both males and females

The inclusion criteria were newly diagnosed patients with cutaneous warts (planter, flat), the age (10-60) years, while the exclusion criteria were**;** dermatological conditions affect vitamins B12 and folic acid (skin hyperpigmentation, vitiligo, angular stomatitis and hair change); medical conditions with b12 and folate deficiency like (pernicious anemia, celiac disease, or inflammatory bowel disease); Vitamin B12 or folate supplements, or take Proton Pump Inhibitors drugs; Pregnancy and lactation; Recent treatment for wart, Sever wart cases (these cases may require more aggressive treatments that can impact overall health and nutrient status); and Immunocompromised individuals (immune status may affect both wart development and nutrient metabolism).

Collection of samples:

Five milliliters of blood were collected from of antecubital fossa of each patient using a (5 ml) disposable syringe, the syringe sample transferred into a gel tube (serum separator tube), and left at room temperature for 20-30 minutes and allowed to clot. The gel tube was then centrifuged at (HEETICH) 3000 RPM (revolutions per minute) for 20 minutes to obtain serum. After collecting the sample, the sample was stored at -20℃. The clear serum was aspirated and divided into 2 aliquots:

1. Aliquote (1): for measurement of B12 was assayed by an automated method by HumaReader HS).

2. Aliquote (2): for measurement of folate was assayed by (HumaReader HS).

Instruments and consumable materials

A. Analytical instrument:

Automatic microliter plate washer and incubator (Combi Wash), and ELISA microplate reader, Semi-automatic, microprocessor-controlled photometer (HumaReader HS).

B. Non-analytical instruments and consumable materials:

Centrifuge (Hettich), disposable tube/Eppendorf tube, serum separating gel tube, syringes 5ml volume with needles, and tourniquet.

C. Biochemistry and immunoassay kits:

The kit is a sandwich enzyme immunoassay for in vitro quantitative measurement of vitamins B12 and FA in human serum, plasma, tissue homogenates and other biological fluids.

Measurement of B12 and folic acid:

They were measured by the clinical chemistry analyzer (Humareader HS) in human serum.

Test principle and Reference interval of Serum B12:

This kit uses enzyme-linked immune sorbent assay (ELISA) based on the Biotin double antibody sandwich technology to assay the Human Vitamin B12 (VB12). Add Vitamin B12(VB12) to the wells, which are pre-coated with Vitamin B12(VB12) monoclonal antibody, and then incubate. After that, add anti-VB12 antibodies labeled with biotin to unite with streptavidin-HRP, which forms an immune complex. Remove unbound enzymes after incubation and washing. Add substrate A and B. Then the solution will turn blue and change into yellow with the effect of acid. The shades of solution and the concentration of Human Vitamin B12 (VB12) are positively correlated (**Lee, Wang et al. 2016**).

ASSAY RANGE : 5pmol/L→1500pmol/L; SENSITIVITY : 2.21pmol/L. The TMB Substrate Wash Buffer (30 × concentrate) and Stop Solution should be stored at 4oC upon receipt, while the others should be at -20oC.

Test principle and Reference interval of Serum FA:

This kit uses enzyme-linked immune sorbent assay (ELISA) based on the Biotin double antibody sandwich technology to assay the Human Folic acid (FA). Add Folic acid (FA)to the wells, which are pre-coated with Folic acid (FA)monoclonal antibody, and then incubate. After that, add anti-FA antibodies labeled with biotin to unite with streptavidin-HRP, which forms an immune complex. Remove unbound enzymes after incubation and washing. Add substrate A and B. Then the solution will turn blue and change into yellow with the effect of acid. The shades of solution and the concentration of Human Folic acid (FA) are positively correlated (**Manual 2020**).

ASSAY RANGE : 0.05nmol/L→20nmol/L; SENSITIVITY : 2.21pmol/L. The TMB Substrate Wash Buffer (30 × concentrate) and Stop Solution should be stored at 4oC upon receipt, while the others should be at -20oC.

Statistical analysis:

The statistical analysis data were analyzed using Statistical Package for Social Sciences (SPSS) version 26, and the data were expressed as means ± SD. Student's t-test was used to compare the mean of two groups, Pearson's correlation test (r) was used to assess the correlation between each vitamin B12 and folic acid, P-value less than or equal to 0.05 was considered statistically significant

Result:

Table 1 shows the age of the study population was successfully well-matched between the groups. However, serum FA and vitamin B12 levels showed a significant reduction ($p \leq 0.05$) in warts patients compared to healthy individuals.

Table 1: Comparison of age, folic acid (FA), and serum vitamin B12 levels between healthy individuals and cutaneous wart patients.

Parameters	Healthy No: 38 Mean ± SD	Wart patients No: 50 Mean ± SD	p-value
Age	27.76 ±11.40	26.34±12.46	P >0.05
S. (V.B12) pmol/L	281.20 ±115.3	40.38± 17.75	0.001
S. (FA) nmol/L	23.49 ±6.25	1.448 ±0.16	0.001

Patients were grouped based on cutaneous wart type to assess whether it had an effect. In Table 2, show non-significant difference was found between the two groups (plantar wart and flat wart) for either folic acid or vitamin B12 levels, as the p-values for both comparisons were greater than 0.05.

Table 2: Comparison of serum folic acid (FA) and serum Vitamin B12 Levels Between the Planter and Flat Groups of wart.

Parameters	Patients with Plantar Wart No: 25 Mean ±SD	Patients with Flat warts No: 25 Mean ± SD	p-value
S. (V.B12) pmol/L	37.59 ±16.73	38.03 ±13.39	P >0.05
S. (FA) nmol/L	1.181 ±0.650	1.268 ± 0.797	P >0.05

Table 3 shows a non-significant correlation ($p > 0.05$) between vitamin B12 and folic acid in healthy individuals. The purpose of determining the levels of these vitamins in healthy individuals is to gather general information about the relationship between them in this population.

Table 3: Correlation between serum vitamin B12 and folic acid Levels in Healthy Individuals

Person's correlation	S.(FA) in healthy nmo/l No:38 r-value	p-value
S. (V.B12) in healthy pmol/l	0.01	0.59

The same result as in the previous correlation was observed in Tables 3 table 4, showing no significant correlation ($p \geq 0.05$) between vitamin B12 and folic acid in cutaneous warts patients.

Table 4: Correlation between serum vitamins B12 and folic acid levels in cutaneous warts patients

Person's correlation	S. (FA) in cutaneous warts patients nmol/l No:50 r-value	p-value
S.(V.B12) in cutaneous warts patients pmol/l	0.082	0.570

Same finding, there is a non-significant correlation between folic acid and B12 in both types of cutaneous warts patients (flat and planar), as Tables 5 and 6.

Table 5: Correlation between serum Vitamins B12 and folic acid levels in flat warts patients

Person's correlation	S.(FA) in flat warts patients No:25	p-value
S.(V.B12) in flat warts patients	0.173	0.408

Table 6: Correlation between serum vitamins B12 and folic acid levels in planter warts patients

Person's correlation	S.VB9 (FA) in planter warts patients N0:25	p-value
S.(V.B12) in planter warts patients	0.165	0.430

Tables (7) and (8) show a significant difference in both serum folic acid and V.B12 levels between the cutaneous warts patient group and the healthy control group when comparing female cutaneous warts patients with healthy females, as well as male cutaneous warts patients with healthy males.

Table 7: Mean ±SD values of serum vitamins B12 and folic acid of the females in the cutaneous warts patient group, and the females in the healthy group.

Parameter	Females with cutaneous warts No. 27	Healthy females No:20	P-value
S.(FA) nmol/L	1.52 ± 0.17	22.53 ±6.72	0.0001
S.(V.B12) pmol/L	47.3 ±15.43	250.6 120.3	0.0001

Table 8: Mean ±SD values of serum vitamin B12 and folic acid of the males in the cutaneous warts patients group, and males in the healthy group.

Parameter	Males with cutaneous warts No. 23	Healthy males No:18	P-value
S.(FA) nmol/L	1.23 ± 0.13	25.72 ±6.12	0.0001
S.(V.B12) pmol/L	47.3 ±15.43	295.3 ±112.4	0.0001

Table 9 shows a significant difference in serum folic acid levels between female and males cutaneous warts patients, but a non-significant difference in serum vitamin B12 levels.

Table 9: Mean ±SD values of serum vitamins B12 and folic acid of the males and females in the cutaneous warts patient groups.

Parameter	Females with cutaneous warts No:27	Males with cutaneous warts No:23	P-value
S.(FA) nmol/L	1.52 ± 0.17	1.23 ±0.13	0.0001
S.(V.B12) pmol/L	47.3 ±15.43	38.71 ±19.23	0.0857

Discussion:

The results of this study provide valuable insights into the serum levels of folic acid (vitamin B9) and vitamin B12 (VB12) in patients with cutaneous warts compared to healthy individuals. A significant decrease in the serum levels of both vitamins was observed in the wart patients, with p-values of 0.001 for both vitamins, indicating a potential deficiency or metabolic disruption in these individuals. The study's findings are in line with Loo SK et al (**Loo and Tang 2014**) in 2014, which has demonstrated that vitamin B9 and B12 deficiencies are commonly found in various skin conditions, including cutaneous warts, potentially due to an underlying systemic health issue or altered immune function (**Romain, Sviri et al. 2016**). Both vitamins play crucial roles in immune response, cell proliferation, and DNA synthesis (**Alwarawrah, Kiernan et al. 2018**), all of which are necessary for maintaining skin health and immune stability. As such, their deficiency may contribute to the chronicity or severity of wart development, possibly influencing the persistence of the condition (**Robert and Brown 2003**)-(**Gombart, Pierre et al. 2020**).

This study also examined the serum vitamin levels in patients with different types of warts, specifically plantar warts and flat warts. No significant difference was found between the two groups regarding folic acid and vitamin B12 levels ($p > 0.05$), suggesting that the type of wart does not have a substantial impact on vitamin levels. This finding is consistent with a prior study, Partearroyo T, et al (**Partearroyo, Úbeda et al. 2013**) in 2013, which indicates that alterations in vitamin profiles in wart patients are likely due to a broader immune-related or metabolic dysfunction, rather than being specifically related to the type of wart. These findings further support the hypothesis that factors influencing vitamin levels in wart patients may be generalized across all forms of cutaneous warts **(Calder, Carr et al. 2020**). Further investigation into the potential common mechanisms underlying the altered vitamin profiles could help clarify the broader implications of vitamin deficiencies in wart development and progression.

Regarding the correlation between folic acid and vitamin B12 levels, no significant relationship was found in either healthy individuals or patients with cutaneous warts ($p > 0.05$). While both vitamins are essential for similar physiological functions, such as cellular metabolism, this non-significant correlation suggests that their metabolism and absorption may be regulated independently or that different mechanisms govern their interaction in the body. A previous study by Lyon P, et al (**Lyon, Strippoli et al. 2020**) in 2020 has also shown similar findings, where folic acid and B12 levels were not directly correlated in various conditions, supporting the notion of independent regulatory pathways for these vitamins.

The observed vitamin deficiencies in patients with cutaneous warts highlight the importance of considering nutritional supplementation or dietary adjustments in managing this skin condition. Given the critical role of folic acid and vitamin B12 in immune function, clinicians should

consider screening for deficiencies in patients with newly diagnosed cutaneous warts (**Abou-Taleb, Abou-Taleb et al. 2019**). Additionally, future studies should investigate whether correcting these deficiencies could help alleviate wart symptoms and improve treatment outcomes. The lack of correlation between the two vitamins suggests that further research may be needed to explore other potential interactions between these vitamins, immune system markers, and skin health, which could provide new therapeutic avenues (**Diotallevi, Campanati et al. 2022**).

A gender-based comparison within the wart patient group revealed a significant difference in serum folic acid levels, with females exhibiting higher levels than males. This finding is consistent with previous research, Mills JL, Dimopoulos A. (**Mills and Dimopoulos 2015**) in 2015, indicating that men may have a higher requirement for

folic acid to achieve similar erythrocyte folate concentrations. In contrast, no significant gender difference was observed in serum vitamin B12 levels, suggesting that both males and females are similarly affected by B12 deficiency in the context of cutaneous warts (**Mills and Dimopoulos 2015**)-(**Collaborators 2017**).

Conclusion:

The results suggest that low levels of folic acid and B12 might be linked to the occurrence of newly diagnosed cutaneous warts, indicating a potential relationship between vitamin deficiencies and cutaneous wart formation. This observation calls for more in-depth studies to explore the role these vitamins play in the development and management of cutaneous warts.

Recommendations:

1. Investigate the potential therapeutic benefits of folic acid and B12 supplementation in patients with newly diagnosed cutaneous warts.

2. Explore the underlying biological mechanisms by which vitamin deficiencies might contribute to newly diagnosed cutaneous wart development.

3. Examine how lifestyle factors such as diet or environmental exposures influence folic acid and B12 levels in individuals with newly diagnosed cutaneous warts.

4. Assess the effectiveness of combining vitamin supplementation with conventional wart treatments to improve patient outcomes.

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Conflicts of interest:

The authors declare that they have no conflicts of interest relevant to this study.

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Legends:

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