Received: February 6, 2025
Accepted: June 10, 2025

Acad Med J 2025;5(2):14-22
UDC:618.148-006.6-022.6:578.827]:577.161.2
DOI:
Original article

PRESENTATION OF VITAMIN D AND FOLATE SERUM LEVELS IN HISTOLOGICALLY VERIFIED CERVICAL INTRAEPITHELIAL LESIONS IN WOMEN

Nakov Ivana[1], Dimitrov Goran[2]

[1]Gynecology and Obstetrics, Primary Policlinic Centre Manolevi, Skopje, Republic of North Macedonia
[2]University Clinic for Gynecology and Obstetrics, Gynecologic Oncology, Faculty of Medicine, Ss. Cyril and Methodius University in Skopje, Republic of North Macedonia
e-mail: serafimova.ivana@yahoo.com

Abstract

Background: Human papillomavirus is the most common sexually transmitted infection and main cause of cervical cancer. Cervical cancer is still one of the leading causes of death in women of reproductive age, so it is very important to prevent HPV infection and its persistence.

Aim: Due to the increased interest in the role of nutrition in prevention of cervical cancer and the overall health burden of HPV infection, we investigated the HPV status, BMI, smoking habits, and the levels of vitamin D and folate in women with histologically verified cervical intraepithelial lesions and in those with normal findings.

Methods: A total of 188 women were included in this prospective study and were divided into 2 groups. The examined group included 107 women and the control group 81 women.

Results: A statistically significant difference in serum levels of vitamin D and folate was obtained between the groups; however, for both micronutrients, the average serum levels were lower in the studied group. Comparing the individual subgroups in the studied population, there were no significant differences, neither in the level of vitamin D nor in folate.

Conclusion: According to the results obtained in our study, we can conclude that higher levels of vitamin D and folate are associated with a lower risk of cervical intraepithelial lesions. Patients with histopathologically verified intraepithelial lesions have significantly lower concentrations of vitamin D and folate.

Keywords: cervical cancer, human papillomavirus, folate, vitamin D, dietary intake

Introduction

Cervical cancer is a serious health problem affecting women of reproductive age worldwide. According to the World Health Organization (WHO), on an annual basis, approximately 660,000 new cases are being diagnosed worldwide, and approximately 350,000 deaths due to cervical cancer will occur in developing countries due to unavailable effective screening, treatment, and no available vaccination program. To these dramatic numbers, a substantial proportion of cervical cancer cases is also presented by women from regions with increased prevalence of human immunodeficiency virus (HIV) or from specific socio-economic areas characterized by sexual habits, where there is gender inequality, particularly in those countries whose population lives in poverty[1,2]. Cervical cancer primarily affects younger women and as a result, of all children who lose their mothers to cancer, approximately 20% lose them to cervical cancer[2].

Human papillomavirus (HPV) is the most common sexually transmitted infection affecting the genital area, skin, and throat. Persistent high-risk HPV infection of cervical cells can cause the development of abnormal cells, which can then develop into cervical cancer (the cause of cervical cancer in up to 95% of cases)[1]. Risk factors for progression to cancer are high oncogenicity of the HPV type, impaired immune status, presence of another sexually transmitted infection, increased number of births, early age at first pregnancy, use of hormonal contraceptives, and cigarette smoking[1-3].

For many years, science has been looking for an answer to the question of whether something can be done in the field of the prevention of cervical cancer. In addition to the introduced cytology-based screenings that have been shown to reduce the number of cervical cancer patients, many dietary antioxidants have received attention in recent years. The intake of vitamin A and vitamin D can act on the early development of cervical cancer and slow it down. Folate intake prevents or inhibits the progression from HPV infection to various grades of cervical intraepithelial neoplasia[4]. Ozgu *et al*., showed that a possible reason for HPV DNA persistence and related cervical intraepithelial neoplasia is the deficiency of vitamin D [5]. Shim *et al.,* and Chu TW reported a significant relationship between vitamin D and cervicovaginal HPV[6,7]. Low concentrations of vitamin D are more often seen in patients with CIN and invasive cervical cancer[8-10]. Vitamin D is essential for the physiology of many organs in the body, and disruption of this regulatory axis is observed in many medical conditions such as osteoporosis, rickets, susceptibility to infections, autoimmune diseases, diabetes, cardiovascular diseases and cancer[11]. The role of vitamin D in the endocrine system has preventive and therapeutic effects against many types of human neoplasms through various mechanisms that include suppression of cell proliferation, increased apoptosis, and reduced angiogenesis. Furthermore, the role of vitamin D and VDR in the prevention of neoplasms of the breast, endometrium, ovary, cervix, vulva and vagina is clear and significant[11-17].

Folate is an important micronutrient for cell chromosomal stability, so antifolate drugs are administered to deliberately cause DNA damage in rapidly replicating cancer cells[18]. Folate depletion causes increased damage to the DNA of cells, and this has been applied in cancer therapy. Numerous studies show that reduced folate concentrations increase the risk of developing cervical cancer[19,20]. A study that included 2000 women conducted by Zhao concluded that low serum folate may increase the risk of CIN progression, and synergy may potentially exist between low serum folate and hrHPV infection to promote CIN development[21]. On the other hand, high folate concentrations, combined with the methylation of the early promoter of HPV-16, are associated with a reduced risk of developing cervical lesions[22] Pathak *et al.,*[19] demonstrated the important role of folates in modulating the risk of cervical cancer and HPV infection, while a meta-analysis that included 22 case-control studies showed that folate deficiency significantly increases the risk of CIN and cervical cancer[22].

Due to the increased interest in the role of nutrition in prevention of cervical cancer and the overall health burden of HPV infection, we investigated the HPV status, BMI, smoking habits, basic laboratory parameters and the levels of vitamin D and folate in women with histologically verified cervical intraepithelial lesions and in those with normal PAP smear.

Material and methods

Study design and participants

This study was designed as a prospective, case-control study in which 188 women were included, divided into 2 groups. The original number was 200 patients, but 12 of them did not meet the criteria. Patients were recruited by their gynecologists. The examined group included 107 women with histologically verified cervical intraepithelial lesions, while the control group consisted of 81 women with normal PAP test, who visited their gynecologists during the same time frame. This study was conducted in the period from June 2023 to February 2024. The inclusion criteria were as follows: histopathologically verified cervical intraepithelial lesion or normal PAP test for the control group, over 18 years old, and not pregnant. The exclusion criteria were pregnancy and suspected pregnancy, patients under 18 years, history of nutritional megaloblastic anemia, hemolytic disease, leukemia, liver diseases, surgical resection of the cervix of the uterus, and female patients who used B vitamins regularly in the last 3 months. All patients had signed informed consent for participation in the study prior to the collection of 10 ml of venous blood for analysis. Vitamin D and folate concentrations were assessed from peripheral blood samples taken by venipuncture in reagent-free tubes. Blood samples were kept at a temperature of 18°-25°C to coagulate, usually for 15-45 minutes, and then centrifuged to provide a sample for analysis. The samples were frozen at -20°C until analysis.

Determination of vitamin D

Before starting the analysis, the sample was pretreated with a reagent containing sodium hydroxide and released the 25-OH vitamin D from the proteins binding it in the serum. During the first incubation, 25-OH vitamin D bound to 25-OH vitamin D-specific monoclonal antibodies immobilized on magnetic beads. Enzyme-labeled 25-OH vitamin D was then added to the reaction mixture, which competed with 25-OH vitamin D for binding to the antibodies incorporated on the magnetic beads. After the second incubation, the magnetic beads were washed to remove unbound enzyme-labeled 25-OH vitamin D and incubated with the fluorogenic substrate 4-methylumbelliferyl phosphate (4MUP). The amount of enzyme-labeled 25-OH vitamin D that bound to the beads is inversely proportional to the concentration of 25-OH vitamin D in the serum. When calibrating the test, a calibration curve was constructed, which was used to calculate the unknown concentration of 25-OH vitamin D in the serum. The concentration of 25-OH vitamin D in the samples was determined using Tosoh Corporation reagents on a TOSOH AIA-900 automatic immune analyzer.

Determination of folate

Before starting the analysis, the sample was pretreated with a reagent containing sodium hydroxide and dithiothreitol and released folate from the proteins with which it was bound in the serum. Folate from the sample competes with enzyme-labeled folate for a limited number of binding sites on fluorescent bovine folate binding protein, which then binds to anti-FITS (fluorescein isothiocyanate) antibodies immobilized on magnetic beads. Magnetic beads were washed to remove unbound enzyme-labeled folate and incubated with the fluorogenic substrate 4-methylumbelliferyl phosphate (4MUP). The amount of enzyme-labeled folate that bound to the beads was inversely proportional to the serum folate concentration. When calibrating the test, a calibration curve was constructed and used to calculate the unknown concentration of folate in the serum. The concentration of vitamin B9 (folate) in the samples was determined using Tosoh Corporation reagents on a TOSOH AIA-900 automatic immune analyzer.

HPV testing

All patients underwent HPV DNA testing. The samples were analyzed as follows: samples were collected using an endocervical cyto-brush while patients were in the lithotomy gynecological position. Then, swabs were placed into sterile test tubes with 0.5 mL of transport medium. Samples were kept at 2-8ºC and genotyped for human papilloma virus (HPV) using the multiple polymerase chain reaction (PCR) method within 7 days.

The approach involved real-time amplification (multiplex PCR) of DNA fragments from HPV genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68, as well as a β-globin gene fragment in one tube. Polymerase chain reaction (PCR) detection relied on the amplification of a pathogen-specific section of the genome using particular primers. In fluorescent end-point PCR, fluorescent dyes were used to identify the amplified product, which was connected to oligonucleotide probes that bound exclusively to it during thermocycling. A multichannel rotor-type fluorometer was specifically constructed to detect fluorescence emission from the accumulated product without reopening the reaction tubes after the PCR run.

Statistical analysis

Data analysis was performed using the statistical analysis software GraphPad Prism 9.0 (USA). The participants of the study were analyzed according to their age, diagnosed cervical intraepithelial lesions (cytologic diagnosis), presence of HPV type(s), and the concentrations of vitamin D and folate. Differences between the groups were tested by using t-test or ANOVA. Differences were considered statistically significant if the level of significance was $p<0.05$.

Results

Demographic data and basic clinical parameters

This study included a total of 188 patients, divided into a study group of 107 patients with histopathologically proven cervical intraepithelial lesions (CIN 1, 2, and 3), and a control group of 81 patients with a normal PAP test. The mean serum vitamin D level in the study group was 11.52 ng/ml, and in the control group 22.39 ng/ml (Figure 1A). According to prior clinical conventions, we categorized serum 25-hydroxyvitamin D levels as follows: <12 ng/mL - indicative of severe vitamin D deficiency; 12-19 ng/mL - indicative of vitamin D deficiency; 20-30 ng/mL - indicative of vitamin D insufficiency; and ≥ 30ng/mL - indicative of vitamin D sufficiency [29], and for folate ≤ 3 ng/mL - folate insufficiency, 3-17 ng/mL - folate sufficiency, and ≥17 ng/mL - folate above level.

When comparing BMI, age and biochemical parameters such as glucose, cholesterol, triglycerides, SE, Hgb, MCV, and thrombocytes, a statistically significant difference was obtained between the two groups, except for vitamin D and folate. An average BMI value of 25.34± 0.4619 kg/m^2 for the tested group and 23.80±0.3370 kg/ m^2 for the control group was obtained. The average value of cholesterol was 5.273±0.0893 mmol/l for the tested group, and 4.961±0.1019 mmol/l for the control group. The average age of the treated group was 45.98±1.230 years, and of the control group 39.31±1.185 years (Table 1).

The average age of patients was 42.18 years, and 48.4% (n=91) of women from the total number were smokers. In the study group, the average age of patients was 39.26 years, and 49.53% (n=53) were smokers. In the control group the average age was 45.97 years, and 46.91% (n = 38) were smokers.

Table 1. Comparison of basic laboratory parameters, concentration of vitamin D and folate in women between the control group and patients with HPV. Results are shown as mean ± SEM, $p<0.05$, statistically significant

Parameter	Mean ± SEM Control (n=81)	Mean ± SEM HPV (n=107)	p-value (t-test, Mann-Whitney)
Age	45.98±1.230	39.31±1.185	*0.0002**
Vitamin D (ng/mL)	22.40±0.8642	11.53±0.6347	*<0.0001**
Folate (ng/mL)	11±0.4022	7.239±0.3300	*<0.0001**
BMI (kg/m^2)	25.34±0.4619	23.80±0.3370	*0.0076**
Glucose (mmol/l)	5.645±0.1736	5.433±0.0918	0.9059
Cholesterol (mmol/l)	5.273±0.0893	4.961±0.1019	0.0089*
Triglycerides (mmol/l)	2.304±1.062	1.160±0.063	0.4753
Thrombocytes (x10^3/μL)	259.6±8.304	263.1±6.869	0.9327
HGB (g/dL)	13.13±0.1459	14.17±1.046	0.8160
MCV (fl)	83.59±0.8147	85.28±0.6125	0.0898
SE	13.06±0.9987	14.96±1.144	0.3616
Fe (μmol/l)	13.04±0.6725	12.32±0.537	0.3984

Vitamin D serum levels

In the group of patients with cervical intraepithelial lesions, 66.35% (n=71) of patients had severe vitamin D deficiency, 17.75% (n=19) had deficiency of vitamin D, 14.95% (n=16) had insufficiency of vitamin D, and only 0.93% (n = 1) had vitamin D sufficiency.

In the control group, 1.23% (n=1) had severe deficiency of vitamin D, 40.74% (n=36) had deficiency of vitamin D, 43.2% (n=38) had insufficiency of vitamin D, and 14.81% (n=13) had sufficient level of vitamin D. A statistically significant difference ($p<0.05$) was observed in the serum vitamin D levels between the control and the study group (CIN1, CIN2, CIN3).

Folate serum levels

In regard to folate levels, 0.93% (n=1) of patients in the study group had a level below the normal range, 96.26% (n=103) a level in the normal range, and 2.8% (n=3) a level above the normal range.

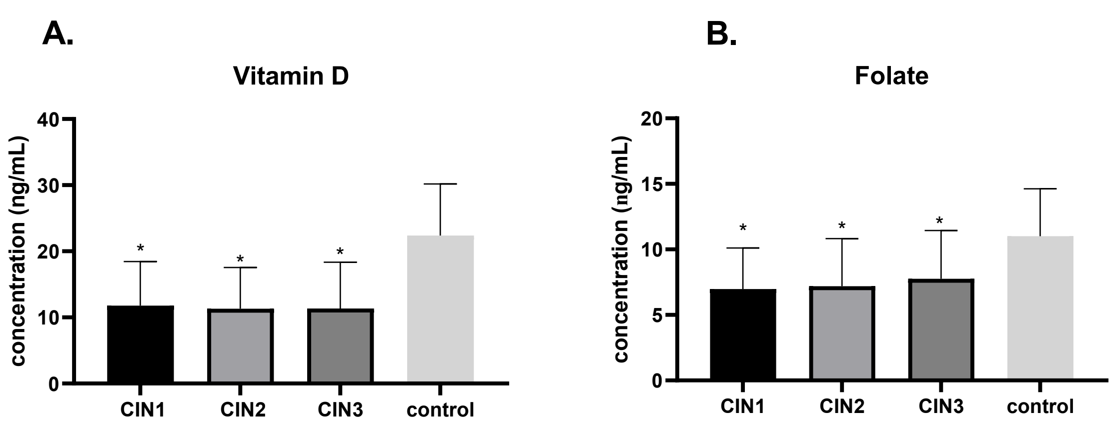

Fig. 1. Mean serum concentration of (A) vitamin D (ng/mL) and (B) folate (ng/mL) in women with cervical intraepithelial lesions and women with normal Pap (control group)

In the control group, 92.59% (n=75) of patients had folate levels in the normal range, and 7.4% (n=6) above the normal range. A statistically significant difference ($p<0.05$) was observed in the serum folate levels between the control and the study group (CIN1, CIN2, CIN3).

For the folate serum levels, the mean value in the study group was 7.24 ng/ml and in the control group 11.0 ng/ml (Figure 1B).

The display of patients according to the cervical intraepithelial lesion and the concentration levels of vitamin D and folate are shown in Table 2.

Table 2. Display of patients according to cervical intraepithelial lesion and concentration levels of vitamin D and folate. Results are shown as Mean ± SEM

Cervical intraepithelial lesion	Vitamin D (ng/ml) ± SEM	p-value (t-test)	Folate (ng/ml) ± SEM	p-value (t-test, Mann-Whitney)
CIN1 (n=47)	11.79±0.97	<0.0001	6.97±0.45	<0.0001
CIN2 (n=33)	11.31±1.08	<0.0001	7.19±0.63	<0.0001
CIN3 (n=27)	11.34±1.35	<0.0001	7.76±0.7	<0.0001
Control (n=81)	22.40±0.86		11.0±0.40	

Of all patients included in this study, 25% had CIN 1 lesions verified histopathologically, and the mean serum level for vitamin D was 11.79±0.97 ng/mL, and for folate 6.97±0.45 ng/mL. Among patients with CIN 2, 17.55% (n=33), the mean serum levels were 11.31±1.08 ng/mL and 7.19±0.63 ng/mL for vitamin D and folate, respectively. In 14.36% (n=27) patients with CIN 3, the results were 11.34±1.35 ng/mL for vitamin D and 7.76±0.7 ng/mL for folate.

In the control group of patients with normal PAP test, the mean level for vitamin D was 22.40±0.86 ng/mL, and the mean concentration of folate was 11.0±0.40 ng/mL (Table 2).

HPV genotype distribution

The distribution of different genotypes of the HPV virus varied among patients.

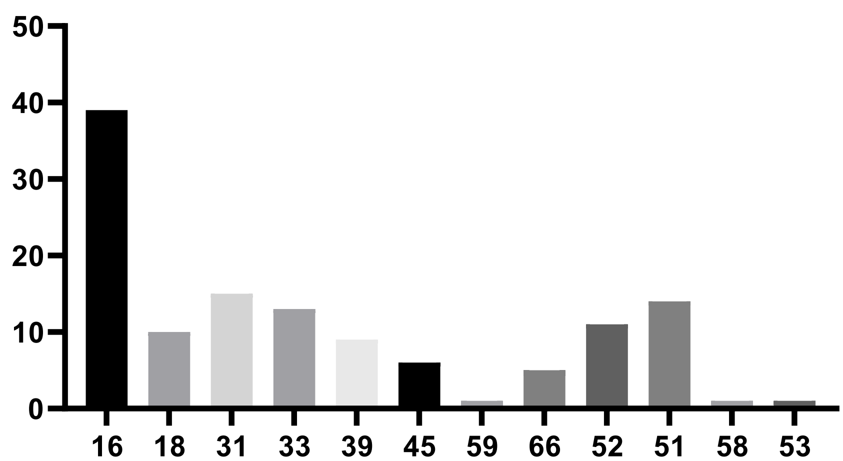

Fig. 2. HPV genotypes according to cytologic diagnosis

As shown in Figure 2, among our respondents, the most common was HPV type 16, followed by HPV type 31, then HPV type 51.

Discussion

Herein presented results confirm the hypothesis of the importance of folate and vitamin D as micronutrients, or rather their deficiency, in the occurrence and progression of pathological conditions that lead to cervical cancer.

Between the studied groups, a statistically significant difference was obtained in the levels of vitamin D in relation to folate; however, for both micronutrients, the average value of the serum levels was lower in the studied group. Comparing the individual subgroups in the studied population, there were no significant differences, neither in the level of vitamin D nor in folate.

In general, a very small percentage, only 6.9% of the total number of patients, had a sufficient serum level of vitamin D. This is, of course, due to sun exposure, and to clothing and eating habits in specific populations. Deficiency of vitamin D is an important public health problem[23-25].

In a randomized controlled trial, patients with cervical intraepithelial neoplasia grade 1 (CIN 1) that received vitamin D for six months *vs*. placebo had higher remission rates[12]. Özgü *et al*. in their study pointed out that the HPV DNA-positive group had lower vitamin D levels than healthy controls defining the relation between HPV infection/cervical intraepithelial neoplasia and vitamin D deficiency. The same study concluded that considering the effects of vitamin D on the immune system, its deficiency might be related to HPV persistence and may cause cervical intraepithelial neoplasia[5].

Shim *et al.,* in their original study examined 2353 sexually active women and pointed out the association of cervical-vaginal HPV prevalence with less-than-optimal levels of serum vitamin D[6].

Numerous studies show that the risk of developing cervical cancer increases when folate concentrations are reduced[19, 20]. A study conducted by Zhao that included 2000 women concluded that low serum folate may increase the risk of CIN progression, and synergy may potentially exist between low serum folate and hrHPV infection to promote CIN development[21].

The methylation of the early promoter of HPV-16 combined with high folate concentrations is associated with a reduced risk of developing cervical lesions[22] Pathak *et al.,* demonstrated the important role of folates in modulating the risk of cervical cancer and HPV infection, while a meta-analysis that included 22 case-control studies showed that folate deficiency significantly increased the risk of CIN and cervical cancer[19]. However, due to the lack of randomized studies over a longer period of time, different doses of supplementation, and different criteria for evaluating CIN, the benefit of folate supplementation in the regression of CIN has not been proven[22].

Vitamin D and its receptors are considered to play a major role in the pathogenesis of gynecological malignancies. Studies have shown that cervical cancer incidence and mortality are inversely correlated with vitamin D levels, and increasing the serum vitamin D helps prevent the risk of cervical neoplasia[10,25,26].

Conclusion

According to the results obtained in our study, we can conclude that higher levels of vitamin D and folate are associated with a lower risk of cervical intraepithelial lesions. Patients with histopathologically verified intraepithelial lesions had significantly lower concentrations of vitamin D and folate. So, correction of vitamin D and folate deficiency is a very important step to improve metabolic parameters since metabolic disorders increase the risk of cervical cancer, and supplementation with them represents a cost-effective benefit for the prevention and treatment of cervical cancers.

Conflict of interest statement. None declared.

References

1. https://www.who.int/news-room/fact-sheets/detail/cervical-cancer.
2. Guida F, Kidman R, Ferlay J, Schüz J, Soerjomataram I, Kithaka B, *et al*. Global and regional estimates of orhans attributed to maternal cancer mortality in 2020. *Nat Med* 2022; 28(12): 2563-2572. doi: 10.1038/s41591-022-02109-2.
3. Stelzle D, Tanaka LF, Lee KK, Ibrahim Khalil A, Baussano I, Shah ASV, *et al*. Estimates of the global burden of cervical cancer associated with HIV. *Lancet Glob Health* 2021; 9(2): e161-e169. doi: 10.1016/S2214-109X(20)30459-9.
4. Tomita LY, Longatto Filho A, Costa MC, Andreoli MA, Villa LL, Franco EL, *et al*. Diet and serum micronutrients in relation to cervical neoplasia and cancer among low-income Brazilian women. *Int J Cancer* 2010; 126(3): 703-714. doi: 10.1002/ijc.24793.
5. Özgü E, Yılmaz N, Başer E, Güngör T, Erkaya S, Yakut Hİ. Could 25-OH vitamin D deficiency be a reason for HPV infection persistence in cervical premalignant lesions? *J Exp Ther Oncol* 2016; 11(3): 177-180. PMID: 28471122.
6. Shim J, Pérez A, Symanski E, Nyitray AG. Association Between Serum 25-Hydroxyvitamin D Level and Human Papillomavirus Cervicovaginal Infection in Women in the United States. *J Infect Dis* 2016; 213(12): 1886-1892. doi: 10.1093/infdis/jiw065.
7. Chu TW, Jhao JY, Lin TJ, Lin TW, Wang CL, Chang HS, *et al*. Vitamin D in gynecological diseases. *J Chin Med Assoc* 2021; 84(11): 1054-1059. doi: 10.1097/JCMA.0000000000000607.
8. Deuster E, Jeschke U, Ye Y, Mahner S, Czogalla B. Vitamin D and VDR in Gynecological Cancers-A Systematic Review. *Int J Mol Sci* 2017; 18(11): 2328. doi: 10.3390/ijms18112328.
9. Yoshizawa T, Handa Y, Uematsu Y, Takeda S, Sekine K, Yoshihara Y, *et al*. Mice lacking the vitamin D receptor exhibit impaired bone formation, uterine hypoplasia and growth retardation after weaning. *Nat Genet* 1997 Aug; 16(4): 391-396. doi: 10.1038/ng0897-391.
10. Hosono S, Matsuo K, Kajiyama H, Hirose K, Suzuki T, Kawase T, *et al*. Association between dietary calcium and vitamin D intake and cervical carcinogenesis among Japanese women. *Eur J Clin Nutr* 2010; 64(4): 400-409. doi: 10.1038/ejcn.2010.28.
11. Heaney RP. Vitamin D in health and disease. *Clin J Am Soc Nephrol* 2008; 3(5): 1535-1541. doi: 10.2215/CJN.01160308.
12. Vahedpoor Z, Jamilian M, Bahmani F, Aghadavod E, Karamali M, Kashanian M, Asemi Z. Effects of Long-Term Vitamin D Supplementation on Regression and Metabolic Status of Cervical Intraepithelial Neoplasia: a Randomized, Double-Blind, Placebo-Controlled Trial. *Horm Cancer* 2017; 8(1): 58-67. doi: 10.1007/s12672-016-0278-x.
13. Vahedpoor Z, Mahmoodi S, Samimi M, Gilasi HR, Bahmani F, Soltani A, Sharifi Esfahani M, Asemi Z. Long-Term Vitamin D Supplementation and the Effects on Recurrence and Metabolic Status of Cervical Intraepithelial Neoplasia Grade 2 or 3: A Randomized, Double-Blind, Placebo-Controlled Trial. Ann Nutr Metab 2018; 72(2): 151-160. doi: 10.1159/000487270.
14. Carlberg C, Velleuer E. Vitamin D and the risk for cancer: A molecular analysis. *Biochem Pharmacol* 2022; 196: 114735. doi: 10.1016/j.bcp.2021.114735.
15. Díaz L, Díaz-Muñoz M, García-Gaytán AC, Méndez I. Mechanistic Effects of Calcitriol in Cancer Biology. *Nutrients* 2015; 7(6): 5020-5050. doi: 10.3390/nu7065020.
16. Shao T, Klein P, Grossbard ML. Vitamin D and breast cancer. *Oncologist* 2012; 17(1): 36-45. doi: 10.1634/theoncologist.2011-0278.

17. Schmidt TT, Sharma S, Reyes GX, Kolodziejczak A, Wagner T, Luke B, *et al*. Inactivation of folylpolyglutamate synthetase Met7 results in genome instability driven by an increased dUTP/dTTP ratio. *Nucleic Acids Res* 2020; 48(1): 264-277. doi: 10.1093/nar/gkz1006.
18. Nazki FH, Sameer AS, Ganaie BA. Folate: metabolism, genes, polymorphisms and the associated diseases. Gene. 2014; 533(1): 11-20. doi: 10.1016/j.gene.2013.09.063.
19. Pathak S, Bhatla N, Singh N. Cervical cancer pathogenesis is associated with one-carbon metabolism. *Mol Cell Biochem* 2012; 369(1-2): 1-7. doi: 10.1007/s11010-012-1362-3.
20. Ragasudha PN, Thulaseedharan JV, Wesley R, Jayaprakash PG, Lalitha P, Pillai MR. A case-control nutrigenomic study on the synergistic activity of folate and vitamin B12 in cervical cancer progression. *Nutr Cancer* 2012; 64(4): 550-558. doi: 10.1080/01635581. 2012.675618.
21. Zhao W, Hao M, Wang Y, Feng N, Wang Z, Wang W, *et al*. Association between folate status and cervical intraepithelial neoplasia. *Eur J Clin Nutr* 2016; 70(7): 837-842. doi: 10.1038/ejcn.2016.35.
22. Yuanxing L, Aimin Y, Wei W, Wang Z, Wang Z, Su X, *et al*. Folic acid intake and folate status and risk of cervical cancer: A systematic review and meta-analysis from 20 independent case-control studies and 4 RCTs. *Curr Dev Nutr* 2020; 4 (Suppl 2): 330. doi: 10.1093/cdn/nzaa044_029.
23. Shim J, Pérez A, Symanski E, Nyitray AG. Association Between Serum 25-Hydroxyvitamin D Level and Human Papillomavirus Cervicovaginal Infection in Women in the United States. *J Infect Dis*. 2016; 213(12): 1886-1892. doi: 10.1093/infdis/jiw065.
24. Mishal AA. Effects of different dress styles on vitamin D levels in healthy young Jordanian women. *Osteoporos Int* 2001; 12(11): 931-935. doi: 10.1007/s001980170021.
25. Granlund L, Ramnemark A, Andersson C, Lindkvist M, Fhärm E, Norberg M. Prevalence of vitamin D deficiency and its association with nutrition, travelling and clothing habits in an immigrant population in Northern Sweden. *Eur J Clin Nutr* 2016; 70(3): 373-379. doi: 10.1038/ejcn.2015.176.
26. Grant WB. The role of geographical ecological studies in identifying diseases linked to UVB exposure and/or vitamin D. *Dermatoendocrinol* 2016; 8(1): e1137400. doi: 10.1080/19381980.2015.1137400.