JOURNAL OF CLINICAL MEDICINE OF KAZAKHSTAN (E-ISSN 2313-1519)
Original Article DOI: https://doi.org/10.23950/jcmk/12113

Serum vitamin D levels in high-risk HPV infected patients, is there any relation?

Anil Turhan Çakir[1], Muhammet Atay Özten[2]

[1]Departments of Obstetrics and Gynecology and Gynecologic Oncology, Faculty of Medicine, Zonguldak Bulent Ecevit University
[2]Department of Obstetrics and Gynecology, Faculty of Medicine, Zonguldak Bulent Ecevit University

Received: 2021-12-28.
Accepted: 2022-04-19

J Clin Med Kaz 2022; 19(3):35-39

Corresponding author:
Anil Turhan Çakir.
E-mail: dranilturhan@hotmail.com;
ORCID: 0000-0001-7976-4123

Abstract

Objective: Human Papilloma Virus (HPV) is a sexually transmittable virus and is the main etiologic cause of uterine cervical cancers. Beyond that, vitamin D is a steroid structured lipid-soluble vitamin, enhancing both humoral and cellular immune responses. In our study, we aimed to investigate the relationship between vitamin D levels and high-risk HPV infections.

Material and methods: A total of 143 patients who applied to the gynecology outpatient clinic between June 2020 and August 2020 were included in the study. Patients with high-risk HPV positivity constituted the study group, and HPV-negative patients constituted the control group. Serum vitamin D levels were compared between the groups.

Results: The mean vitamin D level of all patients included in the study, HPV(+) and HPV(-) patients were 17.57±8.73, 17.54±9.20 and 17.63±7.83, respectively. In the study group 10.2% of the patients and in the control group 8.5% had adequate vitamin D levels. No significant differences in vitamin D levels and in terms of distribution according to categorical vitamin D level have been observed between the groups (p=0.774, p=0.989).

Conclusion: Vitamin D levels were comparable between HPV positive and HPV negative groups, but study and controls both had very low sufficient vitamin D levels. In Turkey and especially in our region (Zonguldak), vitamin D deficiency is almost endemic, and this might be the main reason that both of the groups have very similar vitamin D measurements.

Key words: human papillomavirus, vitamin D, cervix uteri

Introduction

Cervical cancer is the 3rd most common cancer among women all around the world [1]. In economically disadvantaged regions, where cervical cancer screening and prevention programs cannot be financed well, cervix cancer remains the main reason for cancer morbidity and mortality.

Human Papilloma Virüs (HPV) is the most common sexually transmitted disease in adults [2]. Most of the HPV infections were cleared rapidly by natural immunity; however, it may become permanent and cause cancer in some cases [3]. A healthy and stable cellular immunity plays a major role in the eradication of HPV infection of the uterine cervix. HPV is the almost single cause of cervix cancer and is present in 99.7% of cases. Among all high-risk types, HPV 16 is responsible for 50% of the cases, while HPV 18 is present in 20 percent. Other high-risk (HR) types as HPV 31, 33, 45, 52 ve 58 are the cause of the remaining 19 percent [4,5]. HPV positivity was seen in 3.5% in Turkey. The commonest HPV genotypes were 16, followed by 51, 31, 52 and 18 [6]. Cervical cancer estimated age-standardized incidence is 4.8/100.00 and cumulative risk of incidence is %0.78 for Turkey [7].

Vitamin D is considered by many experts as a hormone, lipid-soluble and is in steroid structure and causes many different effects in various tissues [8,9]. Vitamin D plays an important role in immunocompetence. Vitamin D must be in sufficient levels to keep an immune system to prevent any disease, and its deficiency leads to severe disease mortality and morbidity. There is a defined inverse proportionality with vitamin D levels and prolonged upper and lower respiratory tract infections [10]. Studies evaluating cervical-vaginal infections and vitamin D deficiency state that lower vitamin D levels are associated with bacterial vaginosis and chlamydia [11,12].

Vitamin D facilitates the immune modulation of T and B lymphocytes and plays a major role in the natural immune system by proofing them to differentiate in the adaptive immune system, helping monocytes become macrophages, and enhancing their capacity for phagocytosis [13]. Natural immunity is strongly associated with the capacity of producing AMP. AMP, in fact, facilitates the phagocytic cells to react for the infected area and enhances the immune response by regulating the cells responsible for cellular reactions, leading inactivation in pathogens [14]. Vitamin D locally is related to the production of endogen antimicrobial peptides (AMP). In addition, vitamin D helps form a physical barrier, remodeling the proteins of the tight junctions, gap junctions, and adhere junctions to form an extra resistance against bacteria & viruses in the genitourinary system, respiratory system, and the skin [15].

In venereal diseases as HPV, first reaction in the vagina and uterine cervix plays a major role in facing the pathogen in the first place. If the viral capacity could be immunologically beaten off in the first interaction, HPV infection could not persist and could not cause the cervical changes. As mentioned earlier, AMP present in the vagina and cervix plays a major role [16]. An intact barrier is also essential to prevent the transmission of HPV. The proliferation and differentiation process of keratinocytes is regulated by vitamin D. In keeping and maintaining an intact epidermal barrier in the skin, vaginal mucosa, and genitourinary system vitamin D plays a protective and efficient role [17].

So in our study, we wanted to evaluate if the deficiency or the main levels of vitamin D may play a role in the infections with high-risk HPV-subtypes.

Materials and Methods

This study was approved by the ethics committee for clinical studies with the approval number of 2020/12 of Zonguldak Bulent Ecevit University, Turkey. Before the study began, all human participants gave informed consent for HPV testing and for inclusion in the present study. Patients examined in Gynecological Clinic of Zonguldak Bulent Ecevit University between June 2020 - August 2020 has been included to the study. Patients with a positive high-risk screening test have been added to the study group, and other patients with a negative HPV test without cervical complaints formed the controls. Patients' HPV test results and cervical smear screening test results are obtained from the national cervical cancer screening program. In Turkey, women aged between 30 and 65 years are invited for HPV based screening by primary level health staff (family physicians and so called KETEM screening centers) every five years. All screening processes are free of charge. The sample for HPV testing was taken with a brush and put into 5 ml of Standard Transport Medium, HPV DNA specimen collection kits (Qiagen HC2) for HPV DNA analysis. For women who are HPV positive by Hybrid Capture2 (Qiagen), genotyping is performed with the CLART kit (Genomica) [6].

Patients using vitamin D supplementation, with a metabolic disease, liver and kidney disease, or any systemic illness that might affect the vitamin D metabolism and pregnant women are excluded from the study.

A 3ml peripheral venous blood sample was taken from each participant under sterile conditions for the assessment of serum 25-hydroxyvitamin D. Blood samples were collected in labeled tubes and then centrifuged, and the separated sera were stored at -20◦C.

We categorized serum 25-hydroxyvitamin D levels according to prior clinical conventions, as follows: <12 ng/mL, indicative of severe vitamin D deficiency; 12–19 ng/mL, indicative of vitamin D deficiency; 20–29 ng/mL, indicative of vitamin D insufficiency; and ≥30 ng/mL, indicative of vitamin D sufficiency [18].

Statistical analysis of the study was performed using the R 4.0.3 package program. The descriptive statistics of the quantitative variables in the study are given with their mean, standard deviation, median, minimum and maximum values; qualitative variables are reported as frequency and percentage. The conformity of quantitative variables to normal distribution was examined using the Shapiro-Wilk test. The Mann-Whitney U test was used to compare two groups of quantitative variables that did not show normal distribution. Chi-Square Test was used to compare between HPV positive and negative groups according to the categorical distribution of vitamin D. Results less than p=0.05 in all statistical analyses were considered statistically significant.

Results

One hundred forty-three patients fulfilling the admission criteria have been added to the study; among those, 94 HPV negative patients formed the control group, and 49 high-risk HPV positive patients concluded the study group. Patients were between 18-65 ages, and the mean age was 42.54±10.13. Seven of the study group were HPV 16 positive, 33 were HPV other high-risk positive, 6 patients were HPV 16, and HPV other high-risk positive, 2 of them HPV 18 and HPV other high-risk positive and finally 1 patient was HPV 16, HPV 18 and HPV other high-risk positive (Table 1). Considering the cervical smear results, 77 cases had benign results as atrophic cells or infection, 6 patients had Atypical Squamous Cells of Undetermined Significance (ASCUS), 1 had Low-Grade Squamous Intraepithelial Lesions (LSIL) (Table 1).

Table 1 HPV and smear results of patients

	n
HPV Subtypes	
HPV 16	7
HPV Other	33
HPV 16+HPV Other	6
HPV18+HPV Other	2
HPV 16+HPV18+HPV Other	1
Smear Results	
Benign Results (atrophic cells or infection)	77
ASCUS*	6
LSIL*	1

*ASCUS: Atypical Squamous Cells of Undetermined Significance, LSIL: Low-Grade Squamous Intraepithelial Lesions

Table 2 Vitamin D levels of control and study groups

	Mean	Median	Std. Deviation	Minimum	Maximum
HPV+	17.63	16.03	7.83	4.88	35.58
HPV -	17.54	16.34	9.20	3.59	55.02

p=0.774 (Mann-Whitney U test)

Table 3 Distribution of groups according to categorical vitamin D level

	Categorical Vitamin D Level*			
	Severe deficiency	Deficiency	Insufficiency	Sufficiency
HPV-	28 (%29.8)	35 (%37.2)	23 (%24.5)	8 (%8.5)
HPV+	14 (%28.6)	8 (%36.7)	12 (%24.5)	5 (%10.2)

p=0.989 (Chi-Square Test)
*: <12 ng/mL, indicative of severe vitamin D deficiency; 12–19 ng/mL, indicative of vitamin D deficiency; 20–29 ng/mL, indicative of vitamin D insufficiency; and ≥30 ng/mL, indicative of vitamin D sufficiency

The mean vitamin D level of all patients included in the study was 17.57±8.73. When comparing the mean vitamin D levels of controls and study (17.54±9.20 vs. 17.63±7.83, respectfully), there was no statistically significant difference (p=0.774) (Table 2). 28.6% of the study cases had severe vitamin D deficiency, 36.7% were deficient, while 24.5% had insufficient levels of vitamin D. Only 10.2% of the study group had sufficient levels of vitamin D. Similarly, controls had very comparable levels; 29.8% severe deficient, 37.2% deficient, 24.5% insufficient and 8.5% sufficient. No significant difference has been observed between the groups in terms of distribution according to categorical vitamin D level. (p=0.989) (Table 3).

Discussion

Vitamin D plays an active role in the immune system. HPV is eliminated by the immune system. Clearing this agent from the cervix and the factors affecting it are important. Because if it cannot be removed, it can cause cervical precancerous lesions and cancer. Therefore, the relationship between HPV and vitamin D levels is important. In our study, we compared vitamin D levels in HPV positive and HPV negative patient groups. No significant differences in vitamin D levels and in terms of distribution according to categorical vitamin D level have been observed between the groups in our study.

Recent studies brought more interest in the relationship between vitamin D and cancer. Vitamin D and its metabolites' antiangiogenetic effect and its effect on cell proliferation, apoptosis, cell differentiation promote scientific curiosity to this level [19]. Vitamin D and its receptor are considered to play a major role in the pathogenesis of gynecological malignancies. Studies have shown cervical cancer incidence and mortality are inversely correlated with vitamin D levels, and increasing the serum vitamin D helps preventing the risk of cervical neoplasia [20-22]. In a randomized controlled trial, patients with Cervical Intraepithelial Neoplasia grade 1 (CIN1) receiving vitamin D for six months vs. placebo had higher remission rates [23]. Özgü et al., in their research defining the relation between HPV infection/ cervical intraepithelial neoplasia and vitamin D deficiency, pointed out that the HPV DNA-positive group had lower vitamin D levels than healthy controls. 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 [19]. In studies with CIN and vitamin D, Vahedpoor et al. found that the use of vitamin D was effective in mild CIN regression, Schulte-Uebbing et al. demonstrated that vaginal vitamin D supplements had an antidysplastic effect on mild CIN but not on moderate CIN [24,25].

There are few studies evaluating the relation of vitamin D and HPV infection. In a study, patients with venereal warts (condyloma acuminatum) have been associated with lower vitamin D levels [26] but besides that, there are also studies that do not detect a difference [27]. In another research, vitamin D deficiency has been found related with both low-risk HPV infection (1.41; 95% CI 1.23 to 1.61; p<0.001) and high-risk HPV infection (1.25; 95% CI 1.04 to 1.49; p=0.014) [28].

Shim et al., examined 2353 sexually active women in their original research and pointed that cervical-vaginal HPV prevalence is associated with less-than-optimal levels of serum vitamin D. They also calculated per each 10 ng/mL decrease in serum 25(OH)D level the odds of high-risk HPV infection were increased (adjusted odds ratio [aOR], 1.14; 95% confidence interval [CI], 1.02–1.27) [18]. In the Chu at all study, they found a correlation between low vitamin D levels and higher abnormal smear and HPV positivity [29].On the other hand, in another controlled trial, 404 women between ages 30-50 did not have a correlation with lower serum 25(OH)D levels and high-risk HPV infections [16]. In the study in which serum 25-hydroxyvitamin D level and vaginal HPV prevalence, incidence and clearance were evaluated, no relationship was found between vitamin D level and HPV prevalence and incidence, but a modest negative correlation was found in HPV clearance [30]. In the study, evaluated associations between vitamin D biomarkers and persistent high-risk human papillomavirus (hrHPV) detection, among mid-adult women evaluated monthly for 6 months, serum concentrations of 25(OH)D were positively associated with a short-term pattern of persistently detected hrHPV. But associations were inconsistent and significant only in sensitivity analyses [31].

Vitamin D deficiency is an important public health problem, and unfortunately, it is also very common in Turkey. In major studies it has been found that in general population 74,9%, in women in reproductive age 54% was found vitamin D deficient [32,33]. Due to the fact that Turkey is a huge country with various microclimates, vitamin D levels show a huge variability changing in the region. Habitations as İzmir, with moderate weather and more sun exposure vitamin D deficiency, might become as low as 27.8%. In other areas with insufficient sunlight exposure, it may rise to 76.3%-83.4% [26,34]. Studies evaluating vitamin D deficiency have shown that it is very common in our -Zonguldak- region [35,36].

All cases included in our study had mean vitamin D levels of 17.57±8.73. When we look at the mean serum vitamin D levels of controls (17.54±9.20) and study (17.63±7.83), no significant difference has been observed (p=0.774). Spite the fact that we planned our study for the summer period to avoid lower levels of vitamin D, only 10,2% in HPV positive and 8,5% of HPV negative groups had sufficient levels of serum vitamin D. In a similar study from Turkey, Mertoglu et al. did not found any relation concerning vitamin D deficiency and HPV infection, but they too have very few sufficient patients in study and controls (Vitamin D deficiency 82.4% in HPV positive group and 83.4% in HPV negative group). Both groups had a severe deficiency in our study population concerning vitamin D. This might directly affect our results and other Turkish-made studies designed on this topic [26]. More extensive population-based studies, based on vitamin D sufficient and insufficient groups and their HPV screening results, may improve the current knowledge on this topic.

Our study had some limitations. The main limitation of this study was the smaller sample size. The data were collected from a single center. As in previous observational studies, we measured vitamin D levels only at baseline. We used only one vitamin D biomarker (25-hydroxyvitamin D).

Turkey/Zonguldak province, where we performed our study, has endemic low vitamin D levels due to climate factors (less sun exposure), nutritional and clothing habits. We did not find differences in serum vitamin D levels in HPV positive and HPV negative groups, but this may be influenced by the high ratio of vitamin D deficient patients.

Disclosures: There is no conflict of interest for all authors.

Acknowledgements: None.

Funding: None.

Ethics Committee Approval: This study was approved by the Zonguldak Bulent Ecevit University Medical Research Ethics Committee (Approval Number: 2020 / 12) on June 10, 2020 and was conducted in accordance with the Declaration of Helsinki.

References

1. Bruni L, Albero G, Serrano B, Mena M, Gómez D, Muñoz J, Bosch FX, de Sanjosé S. ICO/IARC Information Centre on HPV and Cancer (HPV Information Centre). Human Papillomavirus and Related Diseases in the World. Summary Report 17 June 2019. https://hpvcentre.net/statistics/reports/XWX.pdf (Accessed on June 6, 2021)
2. Weinstock H, Berman S, Cates W Jr. Sexually transmitted diseases among American youth: incidence and prevalence estimates, 2000. *Perspect Sex Reprod Health.* 2004;36(1):6-10. https://doi.org/10.1363/psrh.36.6.04
3. Ho GY, Bierman R, Beardsley L, Chang CJ, Burk RD. Natural history of cervicovaginal papillomavirus infection in young women. N Engl J Med. 1998; 338:423–428. https://doi.org/10.1056/NEJM199802123380703
4. Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, Snijders PJ, Peto J, Meijer CJ, Muñoz N Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J Pathol.* 1999;189(1):12. https://doi.org/10.1002/(SICI)1096-9896(199909)189:1<12::AID-PATH431>3.0.CO;2-F
5. de Sanjose S, Quint WG, Alemany L, et al. Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. *Lancet Oncol*. 2010;11:1048. https://doi.org/10.1016/S1470-2045(10)70230-8
6. Gultekin M, Zayifoglu Karaca M, Kucukyildiz I, et al. Initial results of population based cervical cancer screening program using HPV testing in one million Turkish women. *Int J Cancer.* 2018;142(9):1952-1958. https://doi.org/10.1002/ijc.31212
7. Globocan 2020. https://gco.iarc.fr/today/online-analysis-dual-bars-2).
8. Gois P, Ferreira D, Olenski S, Seguro A. Vitamin D and Infectious Diseases: Simple Bystander or Contributing Factor? *Nutrients*. 2017;9(7):651. https://doi.org/10.3390/nu9070651
9. Wang H, Chen W, Li D, Yin X, Zhang X, Olsen N, et al. Vitamin D and Chronic Diseases. *Aging Dis.* 2017;8(3):346. https://doi.org/10.14336/AD.2016.1021
10. Juzeniene A, Ma LW, Kwitniewski M, Polev GA, Lagunova Z, Dahlback A, et al. The seasonality of pandemic and non-pandemic influenzas: The roles of solar radiation and vitamin D. Int. *J. Infect. Dis.* 2010;14(12):e1099-1105. https://doi.org/10.1016/j.ijid.2010.09.002
11. Hensel KJ, Randis TM, Gelber SE, Ratner AJ. Pregnancy-specifc association of vitamin D deficiency and bacterial vaginosis. *Am. J. Obstet. Gynecol.* 2011;204(1):41.e1-9. https://doi.org/10.1016/j.ajog.2010.08.013
12. He Q, Ananaba GA, Patrickson J, Pitts S, Yi Y, Yan F, Eko FO, Lyn D, Black CM, Igietseme JU, Thierry-Palmer M. Chlamydial infection in vitamin D receptor knockout mice is more intense and prolonged than in wild-type mice. *J Steroid Biochem Mol Biol.* 2013;135:7-14. https://doi.org/10.1016/j.jsbmb.2012.11.002
13. Baeke F, Takiishi T, Korf H, Gysemans C, Mathieu C. Vitamin D: modulator of the immune system. *Curr Opin Pharmacol.* 2010;10(4):482-496. https://doi.org/10.1016/j.coph.2010.04.001
14. Bartley J Vitamin D: emerging roles in infection and immunity. *Expert Rev Anti Infect Ther.* 2010;8:1359–1369. https://doi.org/10.1586/eri.10.102
15. Hewison M, Zehnder D, Chakraverty R, Adams JS. Vitamin D and barrier function: a novel role for extra-renal 1 alpha-hydroxylase. *Mol Cell Endocrinol.* 2004;215:31–38. https://doi.org/10.1016/j.mce.2003.11.017
16. Troja C, Hoofnagle AN, Szpiro A, Stern JE, Lin J, Winer RL. Serum Concentrations of Emerging Vitamin D Biomarkers and Detection of Prevalent High-Risk HPV Infection in Mid-adult Women. *Cancer Epidemiol Biomarkers Prev.* 2020;29(7):1468-1474. https://doi.org/10.1158/1055-9965.EPI-20-0126
17. Piotrowska A, Wierzbicka J, Żmijewski MA. Vitamin D in the skin physiology and pathology. *Acta Biochim Pol.* 2016;63(1):17-29. https://doi.org/10.18388/abp.2015_1104
18. 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. https://doi.org/10.1093/infdis/jiw065
19. 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.
20. Grant WB. An ecological study of cancer incidence and mortality rates in France with respect to latitude, an index for vitamin D production. *Dermatoendocrinol.* 2010;2(2):62-7. https://doi.org/10.4161/derm.2.2.13624
21. Grant WB. Does solar ultraviolet irradiation affect cancer mortality rates in China? *Asian Pac J Cancer Prev.* 2007;8(2):236-242.
22. Hosono S, Matsuo K, Kajiyama H, Hirose K, Suzuki T, Kawase T, Kidokoro K, Nakanishi T, Hamajima N, Kikkawa F, Tajima K, Tanaka H. Association between dietary calcium and vitamin D intake and cervical carcinogenesis among Japanese women. *Eur J Clin Nutr.* 2010;64(4):400-409. https://doi.org/10.1038/ejcn.2010.28

23. 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. https://doi.org/10.1007/s12672-016-0278-x
24. Vahedpoor Z, Jamilian M, Bahmani F, Aghadavod E, Karamali M, Kashanian M, et al. 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;8:58–67. https://doi.org/10.1007/s12672-016-0278-x
25. Schulte-Uebbing C, Schlett S, Craiut I, Antal L, Olah H. Chronical cervical infections and dysplasia (CIN I, CIN II): vaginal vitamin D (high dose) treatment: a new effective method? Dermatoendocrinol. 2014;6:e27791. https://doi.org/10.4161/derm.27791
26. Mertoglu C, Nayki U, Nayki C, Gunay M. The relationship between vitamin D and human papilloma virus infection. *J Clin Anal Med.* 2017;8(6):538-540.
27. Shalaby ME, Hasan MS, Elshorbagy MS, Abo Raya AR, Elsaie ML. Diagnostic and therapeutic implications of vitamin D deficiency in patients with warts: A case-controlled study. J Cosmet Dermatol. 2021;10.1111. https://doi.org/10.1111/jocd.14156
28. Gupta A, Villa A, Feldman S, Citow B, Sroussi H. Site and sex-specific differences in the effect of vitamin D on human papillomavirus infections: analyses of NHANES 2009-2014. Sex Transm Infect. 202;97(1):75-76. https://doi.org/10.1136/sextrans-2020-054466
29. Chu TW, Jhao JY, Lin TJ, et al. Vitamin D in gynecological diseases. *J Chin Med Assoc.* 2021;84(11):1054-1059. https://doi.org/10.1097/JCMA.0000000000000607
30. El-Zein M, Khosrow-Khavar F, Burchell AN, et al. Association of Serum 25-Hydroxyvitamin D With Prevalence, Incidence, and Clearance of Vaginal HPV Infection in Young Women. J Infect Dis. 2021;224(3):492-502. https://doi.org/10.1093/infdis/jiaa758
31. Troja C, Hoofnagle AN, Szpiro A, Stern JE, Lin J, Winer RL. Understanding the Role of Emerging Vitamin D Biomarkers on Short-term Persistence of High-Risk Human Papillomavirus Infection Among Mid-Adult Women. *J Infect Dis.* 2021;224(1):123-132. https://doi.org/10.1093/infdis/jiaa711
32. Hekimsoy Z, Dinç G, Kafesçiler S, Onur E, Güvenç Y, Pala T, Güçlü F, Ozmen B. Vitamin D status among adults in the Aegean region of Turkey. *BMC Public Health*. 2010;10:782. https://doi.org/10.1186/1471-2458-10-782
33. Andiran N, Yordam N, Ozön A. Risk factors for vitamin D deficiency in breast-fed newborns and their mothers. *Nutrition*. 2002;18(1):47-50. https://doi.org/10.1016/s0899-9007(01)00724-9
34. Gür EB, Turan GA, Tatar S, Gökduman A, Karadeniz M, Çelik G, et al. The effect of place of residence and lifestyle on vitamin d defciency in pregnancy: Comparison of eastern and western parts of Turkey. J. Turkish Ger. Gynecol. *Assoc*. 2014;15:149–155. https://doi.org/10.5152/jtgga.2014.13048
35. Sel G, Seyhan Baydağ S, Barut A, Akdemir AY, Özmen Ü, Harma M, Harma Mİ. Gebelerde İlk Trimester Plazma D Vitamini Düzeyleri ile Gestasyonel Diyabet Arasında İlişki Var mı? *Türk Diyab Obez.* 2020;1:36-40. https://doi.org/10.25048/tudod.700771
36. Çakır OM. Low vitamin D levels predict left atrial thrombus in nonvalvular atrial fibrillation. *Nutr Metab Cardiovasc Dis*. 2020;30(7):1152-1160. https://doi.org/10.1016/j.numecd.2020.03.023