

## Could 25-OH vitamin D deficiency be a reason for HPV infection persistence in cervical premalignant lesions?

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Vitamin D is an essential precursor to the steroid hormone calcitriol which mainly regulates calcium homeostasis. Moreover anti-proliferative, pro-apoptotic, anti-angiogenic effects of Vitamin D support the ideas of preventive role in various cancer. This study aimed to determine if there is a relationship between HPV DNA infection and cervical intraepithelial neoplasia and Vitamin D deficiency. As a result of the study the difference of 25-OH Vitamin D3 levels between HPV DNA positive group and the control group were statistically significant ( $p=0,009$ ). According to results of our study, with the proven anti-inflammatory functions of Vitamin D, the deficiency of these molecule and its metabolites can be a possible reason for HPV DNA persistence and related cervical intraepithelial neoplasia.

**KEYWORDS:** Vitamin D, cholecalciferol, HPV, Cervical intraepithelial lesions

### INTRODUCTION

Vitamin D is an essential precursor to the steroid hormone calcitriol (1). The property of being a naturally synthesized substance, make vitamin D defined as an attractive material in cancer prevention and treatment studies. Especially in the last two decades relationship with Vitamin D and cancer progression which is known as “Vitamin D/cancer hypothesis” has attracted the attention of researchers. The basis of this hypothesis is constructed with the experimental detection of anti-angiogenic effect of 25-OH Vitamin D3, the active vitamin D metabolite (2), also several control-

ling effects of Vitamin D Receptor [VDR] on cellular proliferation (3), stimulation of differentiation (4) and apoptosis (5). In recent reviews, the effect of Vitamin D in prevention and treatment of various cancers were discussed. The authors concluded that there are strong data from preclinical and some clinical studies that suggest vitamin D deficiency cause an increase in the incidence of cancer (6). Not only in prevention of cancer, for example Zhang et al (7) evaluated the increased anti neoplastic effect of carboplatin by adding  $1,25(\text{OH})_2\text{D}_3$  to the treatment in in vitro ovarian cell lines. They also pointed out that this antineoplastic effect addition of  $1,25(\text{OH})_2\text{D}_3$  alone to the cultures had no antineoplastic effect.

In animal studies Vitamin D is defined as an effective agent in suppressing and development of colon cancer in murine models (8). Meeker et al showed decreased incidence of cancer development in Helicobacter Bilis inoculated mice with Vitamin D supplementation (9).

In epidemiologic studies the result of Vitamin D and cancer relationship are inconsistent. There are some clinical trials that reveals a strong association between serum Vitamin D levels and colorectal (10, 11), breast (11) and prostate cancer (12). However, Schenk et al (13) reported no such association for prostate cancer risk and vitamin D levels.

The conflicting results can be due to the lack of consensus on definitive reference values for an optimal serum Vitamin D levels in human. In general, 25-OH Vitamin D3 levels below 20 ng/ml is accepted as Vitamin D deficiency, also serum Vitamin D levels above 30 ng/mL is generally accepted as sufficient (14) in clinical trials.

As well known, Human Papillomavirus [HPV] persistence is the key factor for development of pre-malignant and malignant lesions of the cervix. Cellular immunity for eradication of HPV infection in squamous cells of cervix is mandatory. Persistence of HPV in cervical cells leads to structural changes in DNA and inhibition of tumor suppressor genes with these changes are accepted as precursors of cancer development.

According to these findings, we postulated the hypothesis of "Vitamin-D deficiency can be a cause of persistence of HPV infection and this deficiency can cause cervical pre-invasive lesions." The aim of our study is to investigate the association between serum vitamin D levels and high grade cervical lesions in HPV DNA positive women.

## MATERIALS AND METHODS

We conducted a case-control study among patients who had admitted to our hospital's Gynecologic Oncology Outpatient Clinic with the diagnosis of abnormal PAP smear results. HPV DNA positive patients with high grade cervical intraepithelial lesions (High grade intraepithelial lesion (HSIL), Atypical Squamous cells cannot exclude high grade lesions (ASC-H), Cervical carcinoma (SCC), were included to the study as study group. HPV DNA negative patients whose colposcopic investigation and biopsy results were negative for any high grade premalignant intraepithelial lesions were defined as control group. HPV DNA testing for determining HPV status, Colposcopic investigation (cervical biopsy if necessary) were performed to all patients. Blood samples 25-OH Vitamin D3 level investigation were also collected from the participants HPV positive patients without any signs of high grade intraepithelial lesions, patients with negative HPV DNA results but positive for any other intraepithelial lesions in their colposcopic biopsy results, patients under Calcium or Vitamin D treatment for any reasons, patients whose serum samples cannot be obtained are excluded from study. The study was approved by Institutional Review Board of our hospital and informed consent was obtained from each subject.

Blood samples were collected after 8 hours of fasting before colposcopic investigation and collection of sample for HPV DNA analysis and separated into serum and stored at  $-80^{\circ}\text{C}$  until it was analyzed. The study protocol was approved by the Institutional Review Boards for human subject protection. Serum levels of 25-OH Vitamin D3 were measured by using an ELISA Method (Immunodiagnostic AG, Germany). Colposcopic investigations were performed by a single experienced surgeon and cervical samples were col-

lected for HPV DNA before the procedure. After colposcopic investigation, cervical biopsies were obtained from suspected areas. In the absence of suspected colposcopic findings, random biopsies were collected for the investigation.

Means, standard deviations were used for continuous variables and frequencies, percentages were used for categorical variables in comparison of study and control group. Independent-samples t test was used to compare unadjusted means of Vitamin D levels between study and control groups. P value  $<0.05$  was considered to be statistically significant. Statistical Package for the Social Sciences version 17.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis.

## RESULTS

Overall, 85 patients were enrolled to the study, 23 patient were considered as study groups according to their HPV status. 62 patients whose HPV DNA testing and cervical biopsy results were negative, are defined as control group. The mean ages of the participants in study and control groups were  $43,44 \pm 9,7$  and  $43,95 \pm 8,0$  respectively. (Table 1)

The HPV type distribution of the study group is demonstrated in Table 2. According to our data the most common HPV type in the study group is type 16 which is positive in 11 out of 22 patients. Type 18, 31, 35, 39 are the other HPV types which are detected in the study group respectively.

The distribution of PAP smear results of the study group is demonstrated in Table 3. According to these findings LSIL and HGSIL are the most common cervical smear abnormality in HPV DNA positive study group.

Mean of 25-OH Vitamin D3 levels of study and control groups were 8,0857 IU/ml and 11,4720 IU/ml respectively. Medians of two groups were also calculated as 7,21 and 9,4750 respectively. The difference of 25-OH Vitamin D3 levels between study group and the control group was statistically significant ( $p=0,009$ ) (Table 4)

## DISCUSSION

The correlation between serum vitamin D levels and cancer is a favorite subjects that clinicians try to figure out. According to in-vitro and molecular based studies vitamin D can be one of the potential metabolites in cancer prevention.

Mean serum Vitamin D levels have great variability among population, geographic status and seasonal status. In general opinion serum levels of Vitamin below

**Table 1.** Age distribution of study and control groups

Age	Overall (n=84)	HPV (+) (n=22)	HPV (-) (n=62)
Mean	43,57 ± 9,3	43,44 ± 9,7	43,95 ± 8,0
Std. Deviation	9,27918	7,6898	9,8319
Minimum	20	28	20
Maximum	65	65	65

p=0,33

<20 ng/ml defined as vitamin D deficiency. In a previous studies conducted in Turkey, Vitamin D deficiency was found 74.9% of the subjects in general population have 25(OH)D deficiency (15) and in 54% of the women in reproductive age (16).

HPV infection persistence is known as one of main causes of the cervical intraepithelial neoplasia and cervical cancer. Infection seems to be transient in majority of women (17). Only a small group of women (approximately 10%) who have persistent high risk HPV infection have a high risk of progression and development of neoplastic lesions of the cervix (18). In our study, Vitamin D levels of HPV DNA positive women with abnormal cervical smear results are statistically lower than HPV DNA negative women. Investigations reveal that majority of immune system cells like macrophages, lymphocytes and neutrophils have Vitamin D receptor

in their nuclei (19, 20). These findings pointed out the potential role of Vitamin D in immune system.

The course of the cervical cancer resembles Helicobacter infection and cancer development of the gastrointestinal tract. As mentioned previously Meeker et al (9) demonstrates the therapeutic effect of Vitamin D supplementation for Helicobacter Bilis related cancer development in an animal study. The similar results of our study, leads us to suspicion for possible preventive effect of Vitamin D in virus or bacterial derived cancers.

In our study, results also demonstrated that mean serum vitamin D levels were significantly lower in both control and study group that means as a public health manner Vitamin D deficiency can be considered as a problem with a high priority. The overall low levels of vitamin D can also be a limitation of our study with the small sample size. These results prevent a conclusion

**Table 2.** HPV DNA type distribution among study group

	HPV (+) (n=22)	%
HPV Type 16	11	55,1
HPV Type 18	5	22,72
HPV Type 35	2	9,09
HPV Type 39	2	9,09
HPV Type 31	2	9,09

**Table 3.** Distribution of PAP smear results of the study group

	PAP Smear (n=22)	%
ASCUS	2	9,1
LSIL	9	40,9
HGSIL	8	36,4
SCC	1	4,5
ASC-H	2	9,1

**Table 4.** 25-hydroxy vitamin D levels of study and control group

	Overall (n=82)	HPV (+) (n=22)	HPV (-) (n=62)
Mean	10,6045	8,2891	11,4262
Median	8,7650	7,2250	9,3250
Std. Deviation	6,199	3,9680	6,6522
Minimum	3,37	3,54	3,37
Maximum	30,21	18,18	30,21

p=0,009

like “Vitamin D deficiency increases the incidence of cervical intraepithelial neoplasia”. However we believe that, these statistically significant results can pave the way for further investigations with a larger sample size.

## CONCLUSION

In conclusion Vitamin D and relation with cancer development and prevention should be a specific area that deeply should deeply investigate about. Revealing the possible preventive effect of this molecule in carcinogenesis gives us a chance to possess a naturally occurring weapon in cancer therapy and prevention.

## CONFLICTS OF INTEREST NOTIFICATION

The authors do not have a conflict of interest.

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