Serum vitamin-D levels in relation to head and neck cancer

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Background
Vitamin D (VD) and its metabolites reduce the incidence of various cancers, including head and neck squamous-cell carcinoma (HNSCC) by inhibiting tumor angiogenesis, stimulating mutual adherence of cells, and enhancing intercellular communication, thereby strengthening the inhibition of cellular proliferation. Epidemiologic and case–control studies have demonstrated that low VD levels are associated with HNSCC risk. Some studies showed that patients with HNSCC who received VD at the time of diagnosis had diminished peripheral blood and intratumoral levels of immunosuppressive CD34 + cells and increased levels of mature dendritic cells. In addition, they showed the quantitative increase of the immune cells within the HNSCC tissue following VD treatment.

Aim
To investigate VD levels in head-cancer and neck-cancer patients as a step to further elucidate its association with head and neck cancer development, progression, and outcome.

Participants and methods
This study showed that VD deficiency is prominent in patients with head and neck cancer than in controls. Although it may increase patients' risk of therapy-related morbidity and poor outcome, it represents an inexpensive prophylactic and cost-effective option in the therapeutic armamentarium as a synergistic agent to traditional treatment strategies.

Results
The mean VD level in group A was 7.4 ± 2 ng/ml and for group B was 21 ± 6 ng/ml (P=0.043), indicating a significant decrease of VD in group A than in group B. Also, VD-deficiency status was significantly correlated with lower hemoglobin levels and with hepatitis C virus seropositivity in the studied cases.

Conclusion
This study showed that VD deficiency is prominent in patients with head and neck cancer than in controls. Although it may increase patients' risk of therapy-related morbidity and poor outcome, it represents an inexpensive prophylactic and cost-effective option in the therapeutic armamentarium as a synergistic agent to traditional treatment strategies.

Keywords:
head and neck cancer, squamous-cell carcinoma, vitamin D

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Introduction

Head and neck cancer (HNC) are the sixth most common cancer worldwide (around 6%) of all cancer cases [1]. HNC can arise from the epithelium of the upper aerodigestive tract such as the oral cavity, pharynx, larynx, paranasal sinuses, and nasal cavity, as well as the deeper tissues of the bone, salivary glands, and various cell types. Among the HNC, oral squamous-cell carcinoma represents more than 90% of the cancer incidents for which alcohol and tobacco consumption are the main factors followed by the oncogenic human papillomavirus [2,3]. Vitamin D (VD) refers to a group of fat-soluble secosteroids responsible for increasing intestinal absorption of calcium, magnesium, and phosphate, and multiple other biological effects. VD exists as two biologically equivalent forms. VD2 (ergocalciferol) is obtained from dietary vegetable sources or oral supplements; VD3 (cholecalciferol) is obtained either from skin exposure to ultraviolet-B irradiation, or via ingestion of oily fish, fortified foods, or oral supplements [4]. VD and its metabolites reduce the incidence of various cancers, including head and neck squamous-cell carcinoma (HNSCC) by inhibiting tumor angiogenesis, stimulating mutual adherence of cells, and enhancing intercellular communication, thereby strengthening the inhibition of cellular proliferation [5]. Epidemiologic and case-controlled studies have demonstrated that low VD levels are associated with HNSCC risk. Low levels of VD have also been associated with an increased risk of colon and rectal cancer, breast cancer, ovarian cancer, prostate cancer, and esophageal cancers [6].

Some studies showed that patients with HNSCC who received VD at the time of diagnosis had diminished peripheral blood and intratumoral levels of immunosuppressive CD34+ cells and increased levels of mature dendritic cells. In addition, they showed the quantitative increase of the immune cells within the HNSCC tissue following VD treatment [7].

Patients and methods

A total of 50 patients with a histologically verified diagnosis of HNSCC were included in the study. Another 50 age-matched and sex-matched healthy volunteers were included as controls. Patients were selected from the outpatient clinic of the ENT Department of Ain Shams University Hospitals and an informed written consent was taken from all participants. The study protocol was reviewed and approved by the Institutional Research Ethics Board of Faculty of Medicine, Ain Shams University. Clinical data, including age, sex, histopathology, tumor site, and stage, were collected. Exclusion criteria included renal and hepatic insufficiency, heart disease, or terminal cancer. Both groups were subjected to full history taking, thorough clinical examination, and laboratory investigations. Venous blood samples were collected from all participants for complete blood count and VD serum levels. Complete blood count samples were collected in EDTA vacutainers and were run on Sysmex XN-1000 automated five parts differential hematology analyzers (Sysmex America, Inc. 577, Aptakisic Road, Lincolnshire. Illinois, 60069. United States (USA)) followed by PB-smear microscopic examination. Blood samples were centrifuged for 15 min at 1250 g. Subsequently, sera were aliquoted and frozen at −70°C, until the time of the assay. The 25-OH D3 (VD) serum levels were measured by enzyme-linked immunosorbent assay (ELISA) technique using Chemux Bioscience ELISA kit for Human VD3 Immunoassay (Chemux Bioscience Inc.-Bio-Equip. Address: 385 Oyster Point Blvd, South San Francisco, CA 92821. United States (USA).). The kit is a solid-phase ELISA, based on the principle of competitive binding. The microplate washer used for well wash was BioRad PW40 (BioRad, Hercules, California, USA). The ELISA reader used was Awareness Technology Inc. Stat Fax 2100 (Westport, UK). Serum creatinine, blood urea nitrogen, calcium, and phosphorus were measured on AU480 chemistry analyzer (Beckman Coulter Inc., USA).

Statistical analysis

The collected data were revised, coded, tabulated, and introduced to a PC using Statistical Package for Social Science, version 21 (IBM Corp., Armonk, New York, USA) and MedCalc, version 12.5 (MedCalc Software bvba, Ostend, Belgium). Data were presented and suitable statistical tests were done according to the type of data obtained for each parameter. The level of significance was at P value less than or equal to 0.05.

The D'Agostino–Pearson test was used to examine the normality of numerical data distribution. Normally distributed numerical variables were presented as mean and SD, and skewed variables as median and interquartile range. Categorical variables were presented as number (%). Differences between cases and controls as regards quantitative data were compared with the unpaired t test (for normally distributed data), or with the Mann–Whitney test (for non-normally distributed data). The Pearson χ^2 test or Fisher's exact test, when appropriate, was used to compare categorical data. Multivariable regression was used to examine the relation between VD, calcium level, and phosphorus level and HNC, adjusting for the effect of possible confounders. All tests were two tailed.

Results

The study included 100 participants, they were categorized into two groups. Group A comprised 50 patients (40 males and 10 females) with various HNSCC sites. Their ages ranged from 27 to 77 years, with a mean of 53 ± 14 years. Group B comprised 50 sex-matched and age-matched healthy volunteers (32 males and 18 females) as controls. Their ages ranged from 20 to 78 years, with a mean of 42 ± 14 years. Collectively, 22 patients of group A and 28 participants of group B were smokers. There was no significant difference between cases and controls as regards sociodemographic characteristics, except for age where cases were significantly older than controls. Regarding previous medical history of cases, eight (16%) cases were diabetics and eight (16%) were positive for hepatitis C virus (HCV) antibody. The participants' characteristics are shown in Table 1.

Regarding the laboratory parameters for the patients, hemoglobin (Hb) level ranged from 8.6 to 16.3 g/dl, with a mean level of 12 ± 2 g/dl. Platelet count ranged from 128 to 654 × 109/l, with a mean level of 270 ± 161 × 109/l. As for chemical profile, blood urea nitrogen level ranged from 4 to 27 mg/dl, with a mean of 15 ± 8 mg/dl. Serum creatinine level ranged from 0.5 to 1.2 mg/dl, with a mean level of 0.79 ± 0.2 mg/dl. The median serum calcium levels were 10 and 10.8 mg/dl in groups A and B patients, respectively, without any statistically significant difference between both groups (P = 0.974). The

median serum phosphorus levels in groups A and B were 3.7 and 3.1 mg/dl, without any statistically significant difference between both groups ($P = 0.271$).

VD level in group A ranged from 1 to 13 ng/ml with a mean level of 7.4 ± 2 ng/ml and for group B was ranging from 13 to 45 ng/ml with a mean of 21 ± 6 ng/ml with a P value of 0.043, indicating a significant decrease of VD in group-A patients when compared with group B, as shown in Table 2.

According to the site of the tumor: 26 (52%) cases were diagnosed as larynx cancer, thyroid cancer and hypopharyngeal carcinoma were the diagnosis for eight (16%) and six (12%) cases, respectively. Two (4%) cases were diagnosed as salivary-gland cancer, also, the same number was diagnosed as tongue cancer and two (4%) cases with anaplastic carcinoma. Twenty-two (44%) cases had lymphadenopathy and 20 (40%) of them had surgery done (Table 3).

Table 1 Age and demographic characteristics of all studied participants

Parameters	Mean±SD/%		*P*	Significance
	Group A	Group B		
Age (years)	53±13	42±14	0.001*	HS
Sex				
Male	80	64	0.075**	NS
Female	20	36		
Smoking				
Yes	44	56	0.457**	NS
No	56	44		

*Student test. **χ^2 tests.

Table 2 Comparison of vitamin D, serum calcium, and phosphorus levels between cases and controls

Parameters	Mean±SD/median		*P*	Significance
	Group A	Group B		
Ca (mg/dl)	10	10.8	0.974	NS
P (mg/dl)	3.7	3.1	0.271	NS
VD Level (ng/ml)	7.4±2	21±6	0.043	S

Ca, calcium; P, phosphorus; VD, vitamin D. Student *t* test.

Table 3 Clinical characteristics of studied cases

Parameters	*n* (%)
Site of cancer	
Cancer larynx	26 (52.0)
Thyroid cancer	8 (16.0)
Hypopharyngeal carcinoma	6 (12.0)
Cancer tongue	4 (8.0)
Supraglottic carcinoma	2 (4.0)
Parotid cancer	2 (4.0)
Neck swelling	2 (4.0)
Lymph nodes	
No	28 (56.0)
Yes	22 (44.0)
Surgery	
No	30 (60.0)
Yes	20 (40.0)

When studying the correlation between VD levels and various sociodemographic characteristics and the site and type of HNC among the cases, none of the studied parameters showed any statistically significant correlation with VD levels, except for the seropositivity for HCV antibody among cases who showed significantly lower VD levels than seronegative cases as shown in Table 4 and Fig. 1.

On the other hand, the correlation between VD levels and the other laboratory parameters among the studied cases showed no statistically significant correlation, except for cases with lower Hb level who were associated with significantly lower VD levels (Table 5 and Fig. 2).

Discussion

VD and its active form, 1,25-hydroxy-vitamin D (1,25-OHD, calcitriol), have an important role in calcium and bone metabolism. The 25-hydroxyvitamin D, the major circulating form of VD, reflects well the cumulative effects of exposure to sunlight and dietary intake of VD [8]. Epidemiologic, molecular, and cellular studies have implicated a role for VD in differentiation, proliferation, apoptosis, and angiogenesis in many tumor types and, consequently, in development and progression of several cancers. Low levels of VD have been associated with an increased risk of oral cavity and esophageal cancers [7].

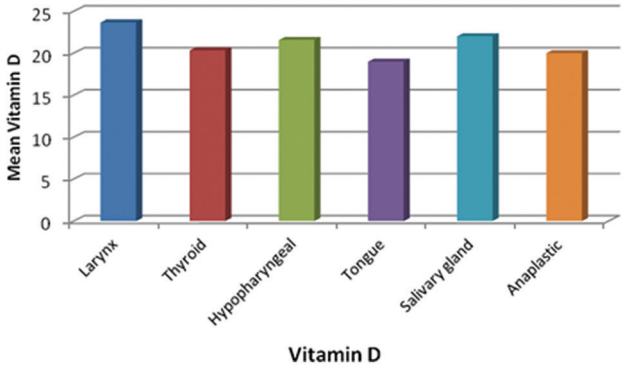
VD has important functions beyond those of calcium and bone homeostasis, which include modulation of the innate and adaptive immune responses. VD receptor is expressed on immune cells (B cells, T cells, and antigen-presenting cells) and these immunologic cells are all capable of synthesizing and responding to VD. VD interaction with the immune system is one of the most well-established nonclassical effects of VD. Supplements of VD in deficient individuals will have beneficial immune-modulator effects on the autoimmune status [9]. It was found that VD can activate the immune system in cancer patients and stimulate immune infiltration within the tumor. Several studies have indeed suggested that adequate VD levels may provide protection against several chronic diseases, including cancer, and could improve cancer prognosis. This was explained by the important and unappreciated biologic function of VD, which is the ability to downregulate hyperproliferative cell growth [10]. The prognostic significance of circulating 25-hydroxyvitamin-D levels among cancer patients has been examined in a limited number of studies [11]. It has been reported that cancer-specific mortality or all-cause mortality was lower for patients with cancer diagnosed during the summer or fall, the seasons with

Table 4 Correlation between vitamin D and demographic characteristics of cases

Parameters	Vitamin D level (mean±SD)	*P*	Significance
Sex			
Male	22.3±7.0	0.457*	NS
Female	19.4±5.0		
Smoking			
Yes	22.2±7.0	0.597*	NS
No	20.9±9.0		
Type of cancer			
Cancer larynx	23.7±19.0		
Thyroid cancer	20.3±4.0		
Hypopharyngeal carcinoma	21.6±6.0	0.974**	NS
Cancer tongue	19.0±1.0		
Salivary gland cancer	22.0±11.0		
Lymph nodes			
Yes	19.9±6.0	0.271*	NS
No	22.6±10.0		
Surgery			
Yes	21.20±9.0	0.163*	NS
No	21.6±8.0		
DM			
Yes	22.0±6.0	0.642*	NS
No	20.7±6.0		
HCV			
Yes	26.3±5.0	0.043*	S
No	20.5±6.0		

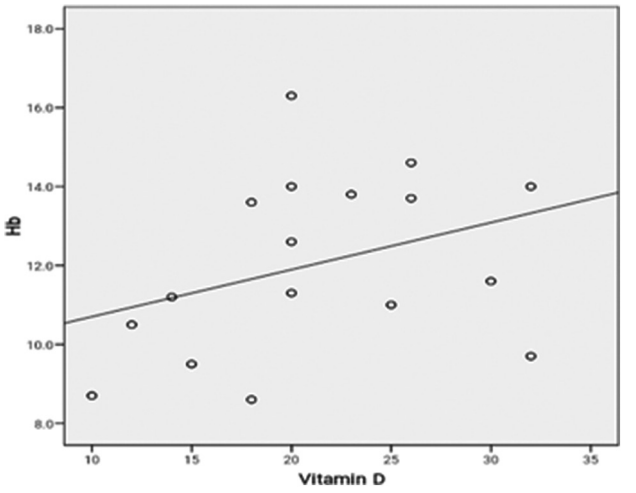
DM, diabetes mellitus; HCV, hepatitis C virus. *Student test. **Analysis of variance.

Figure 1



Patients' clinical characteristics and vitamin-D levels.

Figure 2



Correlations between Hb levels and vitamin D among cases. Hb, hemoglobin.

the highest blood levels of 25-hydroxyvitamin D, than for those diagnosed in the winter [12].

Patients with HNC can experience variable symptoms of depression. Depression has been shown to predict early mortality across a number of different cancers [13].

VD is a unique neurosteroid hormone that may have an important role in many brain processes, including neuroimmunomodulation, regulation of neurotropic factors, neuroprotection, neuroplasticity, and brain development, and it might have a role in psychiatric illness such as depression [14]. Receptors for VD are present on neurons and glia in many areas of the brain, including the promoter regions of serotonin genes, the cingulate cortex, and hippocampus, which have been implicated in the pathophysiology of depression, that its supplementation might play an important part in the treatment of depression [15].

Normal and cancer cells, which have a VD receptor, often respond to VD3 by decreasing their proliferation and enhancing their maturation. In a study, the clinical outcome of VD treatment was also monitored by the time to cancer recurrence. Totally unexpected and most surprising was that the time to cancer recurrence

Table 5 Correlation between age and laboratory parameters and vitamin D level among cases

Parameters	Vitamin D level (Pearson/ Spearman correlation)	*P*	Significance
Age	0.177*	0.220	NS
Hb	0.351*	0.042	S
Platelets	0.033*	0.854	NS
BUN	0.171*	0.403	NS
Creatinine	–0.050*	0.777	NS
Ca	0.254**	0.193	NS
P	0.147**	0.439	NS

BUN, blood urea nitrogen; Ca, calcium; Hb, hemoglobin; P, phosphorus. *Pearson correlation. **Spearman correlation.

following surgical treatment was increased by over threefold in the group receiving VD as opposed to the group of untreated patients [16].

The purpose of this study was to evaluate VD-level status in patients with HNC attending Ain Shams University Hospital. Controversy exists regarding the optimal circulating levels of VD. During recent years, the recommended target serum VD level has been increased based on findings in various intervention and follow-up studies and meta-analyses [16]. The optimal VD level has been defined as 80–100 nmol/l according to Bischoff-Ferrari *et al.*[17] study. Hollis[16] study suggested VD insufficiency level to be less than 40 or 50 nmol/l and values defining VD deficiency were less than 25 nmol/l in a study done by Lips [18]. Buhary *et al.*[19] stated values less than 20 nmol/l as the cutoff for severe VD deficiency.

The present study showed that VD levels were significantly lower in patients with HNC as compared with controls with a *P* value of 0.043. This could be attributed to the fact that patients often have chronic dysphagia and anorexia, and it remains unknown whether VD levels deteriorate as a result of malnutrition or whether preexisting VD deficiency leads to cancer and subsequently to malnutrition.

This result is in accordance with other several studies, which found lower VD levels in cancer patients [20–24]. However, these data still do not support the hypothesis of an association between diet or serum VD level and cancer recurrence or mortality in patients with HNC. There are, to our knowledge, no other published reports investigating the relation of VD to recurrence or survival among HNC patients.

In our study, VD levels were not correlated significantly with age, sex, or smoking status of the studied cases. This could be attributed to the retrospective-study setting and limited number of patients that made it impossible to determine the possible independent role of these risk factors. Concordant results were obtained by the study of Mostafa *et al.* [24].

Elderly people have a higher risk of VD deficiency because of the decreased capacity of the skin to produce adequate amounts of VD, and because of diminished absorption of VD from food products [25]. On the other hand, Orell-Kotikangas *et al.*[26] found higher VD levels in patients 65 years of age or older.

Furthermore, and according to their results, smokers (either at present or during the preceding year) had significantly lower VD levels than nonsmokers (either never-smokers or no smoking during the preceding year). This study concluded that VD insufficiency and deficiency are common in HNC patients at diagnosis. Thus, special attention should be paid to correct the nutritional VD deficiency before treatment. Many authors also recommended that patients with HNC require a substitution of high-dose VD at the start of cancer treatment to bring VD levels to optimum [27,28].

Many authors highlighted a lack of awareness about the importance of VD, worldwide prevalence of VD deficiency, and its management among the general population, especially medical students. This knowledge deficit could provide baseline data to design training modules that would help in identification, prevention, and treatment of VD deficiency. Increased awareness at an early stage could instill adoption of health-related behaviors at personal and professional levels. Effective educational campaigns targeted to specific populations would increase awareness about adequate intake of VD, thereby improving overall health [29].

In the present study, the VD levels were equally poor in patients with various HNC subtypes; this comes in accordance with the results of Mostafa *et al.*[24] and Orell-Kotikangas *et al.* [26]. The present study was not designed and powered to evaluate the role of VD in cancer development and it therefore remains unknown whether the suboptimal VD status may have played a role in cancer development.

Although the significance of poor VD status in the development of HNC is not known, it might have some consequences in the treatment outcome, including occurrence of complications. To our knowledge, there are no data about the role of VD or its analogs in the treatment of head and neck carcinoma. So far, there are no recommendations for optimal serum VD level for patients with cancer. According to Bischoff-Ferrari *et al.*[17] study results, optimal VD levels in cancer prevention might be 80 nmol/l. Satake *et al.*[30] study results supported the idea that vitamin A, D3, and their derivatives are useful for preventing and/or treating patients with HNSCC.

Our study could not detect a significant correlation between the diagnosis of diabetes mellitus among the studied cases and VD levels, but a significant correlation has been elicited between VD levels and HCV seropositivity. This comes in accordance with the results of Jamil *et al*. [31], who concluded that VD deficiency is universal among patients with chronic liver disease, and at least one-third of them suffer from severe VD deficiency.

This opposes the results of Buhary *et al*.[19] who revealed that there is a significant inverse association between serum VD and glycated hemoglobin (HbA1c) levels. An important result of this study highlights a significant reduction in HbA1c as VD levels increased. Advising patients with high HbA1c to get tested for VD serum levels in order to correct any deficiency, may result in better blood-glucose control and benefit the patient's overall health.

The present study has some limitations. The study sample was small in size, and the number of patients in each cancer subtype was not big enough to detect possible differences between the subgroups. Besides, we were not able to assess causality between VD-deficiency status, age, sex, smoking, medical status, or different laboratory investigations and cancer. Furthermore, due to the lack of prospective follow-up, we were not able to determine how VD status could have an impact on treatment outcome. Larger prospective studies with a larger number of patients and longer follow-up periods are needed to establish the significance of VD deficiency on patients' outcome. Further studies are required to elucidate the role of VD deficiency in tumor development and to determine the role of VD and its analogs in treatment outcome in patients with HNC.

Conclusion

In this study, we found that HNC patients had significantly lower VD levels and that these VD levels were not significantly correlated with the different types of HNC. But as VD levels were significantly associated with lower Hb levels and with HCV seropositivity among the studied cases, we concluded that VD deficiency may increase patients' risk for therapy-related morbidity and poor outcome. It may represent an inexpensive prophylactic and cost-effective option in the therapeutic armamentarium as a synergistic agent to traditional treatment strategies.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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