Value of vitamin D assessment in patients with head and neck squamous cell cancer before treatment

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Introduction
The 5-year survival of patients with head and neck squamous cell carcinoma (HNSCC) has not changed dramatically over the last decades despite the use of various therapeutic modalities, including surgery and/or chemoradiation. Thus, new approaches remain necessary to prevent cancer, reduce recurrence, and improve treatment with reduction of its side effects. There is recent evidence that vitamin D promotes cell differentiation and decreases cell proliferation, invasion, angiogenesis, and metastasis. Thus, it has been hypothesized that vitamin D may protect against cancer at multiple sites.
Objective
The aim of this study was to evaluate serum level of vitamin D (VD), calcium, and phosphate in patients with HNSCC before treatment as a step in studying its impact on HNSCC development and progression.
Type of the study
This prospective study was conducted between March 2013 and October 2014 at Ain Shams University Hospitals, Cairo, Egypt.
Patients and methods
The study included 80 participants categorized into two groups. Group A included 50 (36 males and 14 females) patients with various HNSCC sites; their median age was 54.8 ± 12.7 years. Group B included 30 (20 males and 10 females) sex-matched and age-matched healthy volunteers as controls; their mean age was 50.5 ± 12.0 years.
Results
The median VD level in group A was 40.35 (31.9–55) and for group B it was 118.75 (55.0–175) ($P < 0.001$), indicating a significant decrease of VD in group A than in group B. VD deficiency (<37.5 nmol/ml) in group A was 42%, which was significantly more than that in group B, which was only 3%.
Conclusion
This study showed that vitamin D deficiency is prominent in patients with head and neck squamous cell carcinoma before treatment than in controls. Although it may expose the patients to increased risk of therapy-related morbidity and poor outcome, it may constitute an inexpensive prophylactic and cost-effective option in the therapeutic armamentarium as a synergistic agent to traditional treatment options.

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

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Introduction

The 5-year survival of patients with head and neck squamous cell carcinoma (HNSCC) has not changed dramatically over the last decades despite the use of various therapeutic modalities, including surgery and/or chemoradiation [1]. Thus, new approaches remain necessary to prevent cancer, reduce recurrence, and improve treatment with reduction of its side effects.

Vitamin D supplementation is becoming increasingly popular for its role in calcium (Ca) metabolism, muscular function, and prevention of autoimmune and cardiovascular diseases. Whether or not vitamin D metabolites have an impact on cancer development has been controversial and has been recently reviewed [2,3].

There is recent evidence that vitamin D promotes cell differentiation and decreases cell proliferation, invasion, angiogenesis, and metastasis [1,4]. Thus, it has been hypothesized that vitamin D may protect against cancer at multiple sites. Experimental study on mice showed

that therapy with 1,25-dihydroxyvitamin vitamin D_3 [1,25(OH)2D_3] can reduce the extent of prostatic metastatic disease [5]. Other studies have shown evidence supporting a protective association of vitamin D supplementation with colorectal cancer and that it reduces the risk of developing breast cancer, but evidence concerning other cancers is inconsistent [1,6].

Some studies have attributed the anticancer effects of vitamin D to inhibition of proliferation [6–8], whereas the mechanism by which 1,25(OH)2D_3 exerts its antitumor effects is still a myth [9,10].

Other studies showed that 1,25(OH)2D_3 can activate the immune system in cancer patients and stimulate intratumoral immune infiltration [11], it can also modulate tumor cell growth, and apoptosis [12,13]. Among these are the immune regulatory activities of vitamin D, which can impact HNSCC development and progression.

The aim of this study was to evaluate vitamin D (VD) serum concentrations, serum Ca, and phosphate (P) in patients with HNSCC before treatment as a step in studying its impact on HNSCC development and progression.

Materials and methods

This prospective study was conducted between March 2013 and October 2014 at Ain Shams University Hospitals, Cairo, Egypt. The institutional Research Ethics Board reviewed and approved the study.

Only patients with histologically verified new diagnosis of HNSCC were included. Patients with serious renal insufficiency, hepatic insufficiency, and serious heart insufficiency or terminal phase of cancer were excluded. All patients signed a written informed consent before participating in the study.

All patients were evaluated by a multidisciplinary tumor board and after the diagnosis received either surgery or combined treatment consisting of surgery and postoperative radiochemotherapy or radiotherapy alone, as per the established protocols of our center.

A total of 50 consecutive patients fulfilling the inclusion criteria were included in the present study. Clinical details including age, sex, histopathology, and tumor site and stage were collected. Another 30 age-matched and sex-matched healthy volunteers were included as controls.

All measurements were obtained in the same laboratory, by the same researcher, and with the same kit. The blood samples were centrifuged for 15 min at 1250 *g*. Subsequently, sera were aliquoted and frozen at −70°C until the day of assay.

Serum levels of 25(OH)D_3 (vitamin D) were measured by enzyme-linked immunosorbent assay (ELISA) technique using CALBIOTECH ELISA kit for Human VD_3 Immunoassay (catalog no: VD220B; Calbiotech, Spring Valley, California, USA). The kit is a solid phase ELISA, based on the principal of competitive binding. The test results were reported in nmol/l. BioRad PW40 (BioRad, Hercules, California, USA), well washer was used as the microplate washer in the ELISA assessment of serum levels of VD_3. Awareness Technology Inc. Stat Fax 2100 (Westport, UK) was used as the ELISA reader.

Serum Ca and PO_4 parameters were measured on Synchron CX-9 auto-analyzer (Beckman Instruments Inc., Fullerton, California, USA).

Statistical methods

Data were analyzed using IBM SPSS Statistics, version 21 (IBM Corp., Armonk, New York, USA) and MedCalc, version 12.5 (MedCalc Software bvba, Ostend, Belgium).

The D'Agostino–Pearson test was used to examine the normality of numerical data distribution. Normally distributed numerical variables were presented as mean (SD), and skewed variables as median (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). Differences among multiple lesion sites were compared using the Kruskal–Wallis test. 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 vitamin D, Ca level, and P level and head and neck cancer, adjusting for the effect of possible confounders. All tests are two tailed. *P* value less than 0.05 was considered statistically significant.

Results

The study included 80 participants; they were categorized into two groups. Group A comprised 50 patients (36 males and 14 females) with various HNSCC sites, and with a median age of 54.8 ± 12.7 years. Group B

comprised 30 (20 males and 10 females) sex-matched and age-matched healthy volunteers as controls, and with a mean age of 50.5 ± 12.0 years. In total, 37 participants of group A were smokers while 24 of the control group (group B) were smokers. The patient characteristics are shown in Table 1, while the site and stage of head and neck cancer are shown in Table 2.

The median vitamin D (VD) level in group A was 40.35 (31.9–55) and for group B it was 118.75 (55.0–175); *P* value was less than 0.001, indicating a significant decrease of VD in group A than in group B.

Table 1 Patients' characteristics

Variable	Control (*N* = 30)	HNC (*N* = 50)	*P* value
Age (years)	50.5 (12.0)	54.8 (12.7)	0.186[a]
Male/female	20/10	36/14	0.614[b]
Smoker/nonsmoker	24/6	37/13	0.542[b]

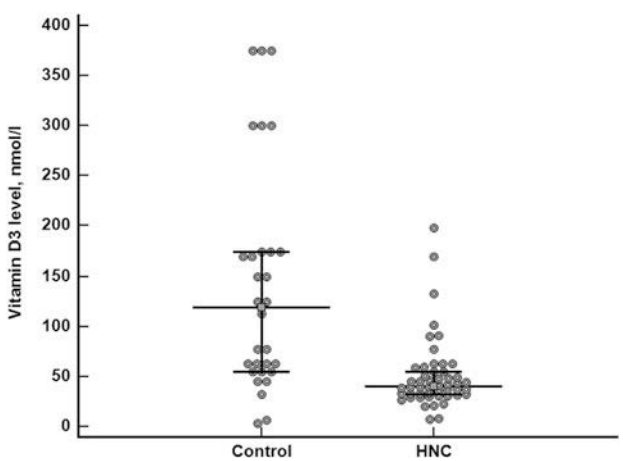
Data are presented as mean (SD) or ratio. [a]Unpaired *t*-test. [b]Pearson χ^2-test. HNC, head and neck cancer.

Table 2 Site and stage of head and neck tumors

Variable	Metric
Site of tumor	
Larynx	19 (38.0)
Hypopharynx	8 (16.0)
Nasopharynx	8 (16.0)
Nasal sinus	4 (8.0)
Oral cavity	9 (18.0)
Unknown primary	2 (4.0)
Stage of tumor	
Stage II	6 (12.0)
Stage III	31 (62.0)
Stage IV	13 (26.0)

Data are presented as *n* (%).

Figure 1



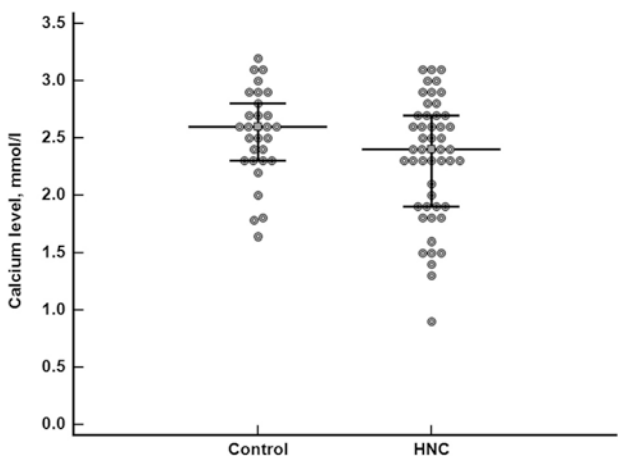
Vitamin D_3 level in cases with HNC and controls. Circular markers represent individual observations. Squared marker with traversing horizontal line represents the median (second quartile). Error bars represent the 25th and 75th percentiles (first and third quartiles, respectively). HNC, head and neck cancer.

VD deficiency (<37.5 nmol/l) in group A was 42% and it was significantly more than that in group B, which was only 3% (Fig. 1). In addition, the optimal VD level (>80 nmol/l) in group A was 6% and this was less than that in group B, which was 16%.

The median serum Ca levels in group A and group B were 2.4 (1.900–2.70 mmol/l) and 2.6 (2.300–2.80 mol/l), respectively; *P* value was 0.097, indicating no statistically significant differences between the two groups (Figs. 2 and 3).

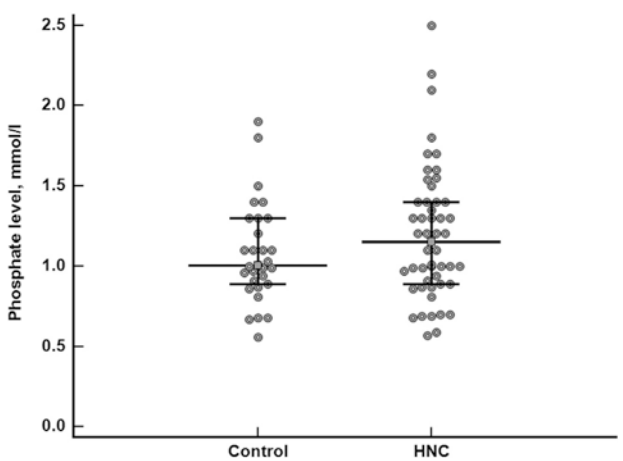
The median serum P level in group A and B were 1.15 (0.89–1.40 mmol/l) and 1.01 (0.89–1.30 mmol/l),

Figure 2



Calcium level in cases with HNC and controls. Circular markers represent individual observations. Squared marker with traversing horizontal line represents the median (second quartile). Error bars represent the 25th and 75th percentiles (first and third quartiles, respectively). HNC, head and neck cancer.

Figure 3



Phosphate level in cases with HNC and controls. Circular markers represent individual observations. Squared marker with traversing horizontal line represents the median (second quartile). Error bars represent the 25th and 75th percentiles (first and third quartiles, respectively). HNC, head and neck cancer.

respectively; *P* value was 0.223, which reflects no statistical significant decrease in the serum PO_4 level in the two groups. The results of the quantitative assay of serum vitamin D, Ca, and P levels in the two studied groups is shown in Table 3. There was no statistically significant difference between serum VD, Ca, and P level concentrations in various tumor sites (Table 4 and Figs. 4–6).

Multivariable regression for determinants of VD, Ca, and P levels was used to examine the effect of possible confounders (smoking, age, and sex), and the results are shown in Table 5. The only significant differences between both groups were in the serum levels of VD.

Discussion

Studies imply that VD contributes to cancer pathogenesis, progression, and outcome. It was found that VD can activate the immune system in cancer patients and stimulate intratumoral immune infiltration [12]. Experimental studies have shown that VD_3 has clinical effectiveness in a hamster buccal pouch tumor model [3]. VD_3 therapy can reduce the extent of metastatic disease and, when combined with adoptive immunity, reduces metastasis [5]. Other studies attributed the anticancer effects of vitamin D to inhibition of proliferation [7,9–11,14].

Our prospective study showed that serum level of VD in patient with HNSCC is significantly lower than that

Table 3 Quantitative assay of serum vitamin D, calcium, and phosphate levels in the two study groups

Variable	Control (*n* = 30)	HNC (*n* = 50)	*P* value
Vitamin D level (nmol/l)	118.75 (55.00–175.00)	40.35 (31.90–55.00)	<0.001[a]
Calcium level (mmol/l)	2.6 (2.300–2.80)	2.4 (1.900–2.70)	0.097[a]
Phosphate level (mmol/l)	1.01 (0.89–1.30)	1.15 (0.89–1.40)	0.223[a]

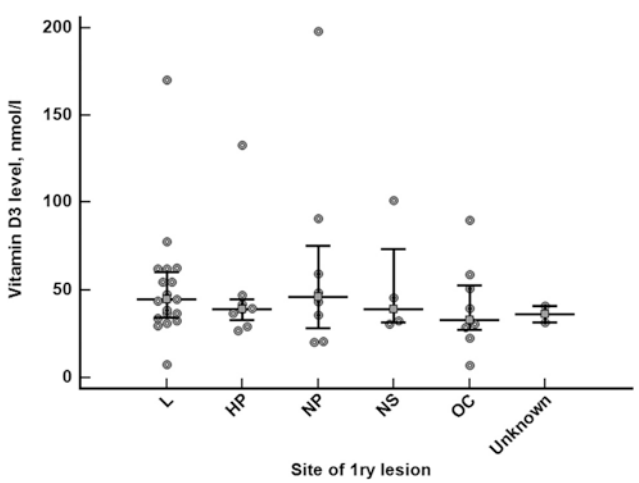
Data are presented as median (interquartile range). [a]Mann–Whitney test. HNC, head and neck cancer.

Table 4 Quantitative assay of serum vitamin D, calcium, and phosphate levels by site of HNC

Variable	Larynx (*n* = 19)	Hypopharynx (*n* = 8)	Nasopharynx (*n* = 8)	Nasal sinus (*n* = 4)	Oral cavity (*n* = 9)	Unknown primary (*n* = 2)	*P* value
Vitamin D_3 level (nmol/l)	45.00 (34.63–60.63)	39.40 (33.20–44.75)	46.10 (28.35–75.30)	39.25 (31.85–73.50)	32.90 (27.40–52.90)	36.40 (31.90–40.90)	0.874[a]
Calcium level (mmol/l)	2.50 (2.30–2.70)	1.95 (1.60–2.60)	2.35 (2.10–2.50)	2.40 (2.30–2.55)	2.30 (1.88–2.78)	2.35 (1.60–3.10)	0.749[a]
Phosphate level (mmol/l)	1.20 (0.96–1.55)	1.45 (1.00–1.90)	1.15 (0.93–1.35)	1.30 (1.25–1.35)	0.89 (0.82–1.00)	0.95 (0.91–0.99)	0.097[a]

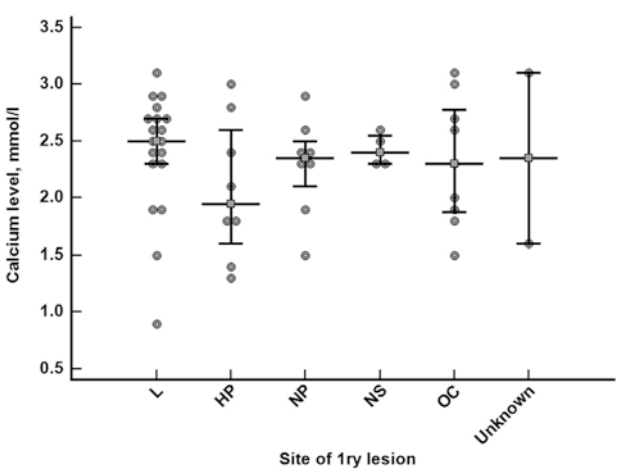
Data are presented as median (interquartile range). [a]Kruskal–Wallis test. HNC, head and neck cancer.

Figure 4



Vitamin D_3 level by site of HNC. Circular markers represent individual observations. Squared marker with traversing horizontal line represents the median (second quartile). Error bars represent the 25th and 75th percentiles (first and third quartiles, respectively). HNC, head and neck cancer; HP, hypopharyngeal; L, laryngeal; NP, nasopharyngeal; NS, nasal sinus; OC, oral cavity.

Figure 5



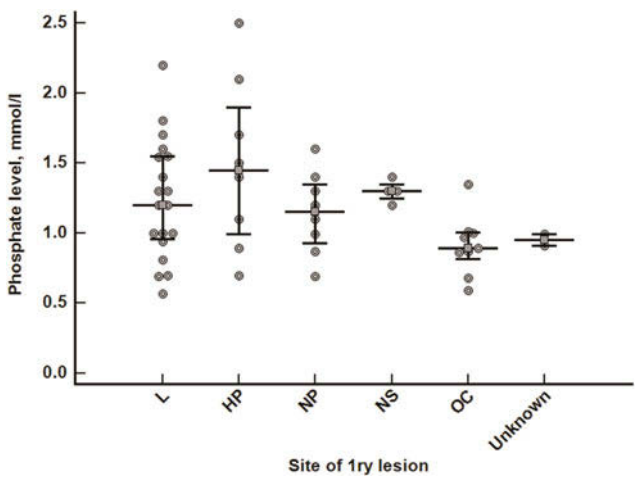
Calcium level by site of HNC. Circular markers represent individual observations. Squared marker with traversing horizontal line represents the median (second quartile). Error bars represent the 25th and 75th percentiles (first and third quartiles, respectively). HNC, head and neck cancer; HP, hypopharyngeal; L, laryngeal; NP, nasopharyngeal; NS, nasal sinus; OC, oral cavity.

Table 5 Multivariable regression for determinants of vitamin D_3, calcium, and phosphate levels

Dependent variable	Independent variables	*B*	SE	$r_{partial}$	*t*	*P* value
Log vitamin D_3 level	Group (control = 0, HNC = 1)	−0.335	0.084	−0.418	−3.986	<0.001
	Age (years)	−0.003	0.003	−0.106	−0.923	0.359
	Sex (female = 0, male = 1)	0.085	0.087	0.112	0.976	0.332
	Smoking (nonsmoker = 0, smoker = 1)	0.083	0.094	0.101	0.879	0.382
	Constant	2.003				
Log calcium level	Group (control = 0, HNC = 1)	−0.046	0.024	−0.219	−1.942	0.056
	Age (years)	0.000	0.001	−0.034	−0.293	0.770
	Sex (female = 0, male = 1)	0.026	0.025	0.122	1.064	0.291
	Smoking (nonsmoker = 0, smoker = 1)	0.005	0.027	0.020	0.173	0.863
	Constant	0.390				
Log phosphate level	Group (control = 0, HNC = 1)	0.043	0.032	0.152	1.335	0.186
	Age (years)	0.000	0.001	−0.006	−0.050	0.961
	Sex (female = 0, male = 1)	−0.028	0.034	−0.096	−0.839	0.404
	Smoking (nonsmoker = 0, smoker = 1)	0.057	0.036	0.179	1.579	0.119
	Constant	−0.008				

HNC, head and neck cancer.

Figure 6



Phosphate level by site of HNC. Circular markers represent individual observations. Squared marker with traversing horizontal line represents the median (second quartile). Error bars represent the 25th and 75th percentiles (first and third quartiles, respectively). HNC, head and neck cancer; HP, hypopharyngeal; L, laryngeal; NP, nasopharyngeal; NS, nasal sinus; OC, oral cavity.

of controls with a *P* value of less than 0.001. This result was in accordance with several studies, which found low vitamin D concentrations in patients with cancer [15–18].

Orell-Kotikangas and colleagues concluded in their study that vitamin D insufficiency and deficiency are prevalent in patients with head and neck cancer at diagnosis. Therefore, special attention should be paid to correct the nutritional deficits, especially vitamin D deficiency, before treatment [14]. Many authors also found that there are extremely low levels of VD in patients with HNSCC and they recommended that patients with head and neck cancer would need a high dose of VD substitution at the start of cancer treatment to bring VD values to the optimal level. Other studies showed the important role of VD in the incidence, prevention, survival, and treatment of several cancers [19–22]. They showed promising results with the use of VD as an anticancer drug in colon, prostate, and hepatocellular cancers [23–25].

This was explained by the important and unappreciated biologic function of VD, which is the ability to down-regulate hyperproliferative cell growth [26,27]. Normal and cancer cells, which have a vitamin D receptor, often respond to VD_3 by decreasing their proliferation and enhancing their maturation [27]. Nowadays, it is well known that the immune depression in HNSCC patients is caused by immune suppressive mediators produced by the HNSCC cells and the immune suppressive cells that they induce [28].

Some studies showed that the enhancement of dendritic cell differentiation from CD34+ cells (+ progenitor cells to HNSCC-induced immune dysfunction) of HNSCC patients supported determining whether treatment of HNSCC patients with VD_3 would reduce levels of CD34+ immune suppressive cells, increase the levels of mature dendritic cells, and increase the levels of stimulated T-cells. These studies showed that patients with HNSCC who received VD_3 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_3 treatment.

In a study, the clinical outcome of VD treatment was also monitored by the time to cancer recurrence [28]. Totally unexpected and most surprising was that the time to cancer recurrence following surgical treatment was increased by over three-fold in the group receiving VD as opposed to the group of untreated patients [29–33].

It was found that vitamin D could enhance immune reactivity by other immunological means. It can diminish levels of cyclooxygenase-2 (COX-2) [34–36]. For example, VD can normalize overexpressed levels of COX-2 in estrogen-deficient rats [37]. Overexpression of COX-2 by both HNSCC as well as several immune inhibitory cell types that they induce contributes to the immune dysfunction in HNSCC patients [38–41], as tumor-associated macrophages [41] and endothelial cells, which we recently identified to be induced by HNSCC [42]. Studies have shown immune restoration and prolonged survival associated with COX-2 inhibition [43,44].

Although the significance of poor VD status in the development of head and neck cancer is still not known, the precise nature of the interaction between VD and the immune system remains unresolved. It might have some consequences in the treatment outcome, including occurrence of complications [45,46].

There were no statistically significant differences as regard Ca level between group A and group B. A large study conducted by Need and Nordin [47] also showed no relation between serum VD concentrations and Ca level; the investigators proposed that VD deficiency does not reduce Ca absorption. Several other studies also showed no relation between VD concentrations and Ca absorption in children and adolescents [48,49]. Aloia *et al.*[50], in their study, concluded that the relation of serum VD concentrations to Ca absorption cannot be used as a biomarker for vitamin D sufficiency. A systematic review by Chung *et al.*[51] concluded that for many outcomes it was difficult to draw firm conclusions on the basis of the available literature concerning the association of both serum VD concentration and Ca level.

In addition, there were no statistically significant differences as regards P level between group A and group B. This was observed by Brot *et al.*[52]. However, Abrams and colleagues found a significant decrease in the serum PO_4 level in patients with VD deficiency, a result that could be explained by the following: vitamin D deficiency is known to stimulate the parathyroid glands, leading to secondary hyperparathyroidism. This maintains serum Ca in the normal range at the expense of mobilizing Ca from the bone and increases urinary P loss resulting in a decrease in serum P level [49].

Our study showed that VD levels were not influenced by gender, site, and age in both groups. This is in accordance with the study conducted by Apuhan *et al.*[53]. However, Orell-Kotikangas *et al.* [14], in their study, found higher vitamin D levels in patients 65 years of age or older. Other studies showed that older age and female sex are associated with an increased risk of being vitamin D deficiency [54]. Elderly people have a higher risk of vitamin D deficiency because of the decreased capacity of the skin to produce adequate amounts of vitamin D, and because of diminished absorption of vitamin D from food products [55,56].

There was no significant statistical difference between VD level and smoking habit in both groups. On the contrary, Orell-Kotikangas *et al.*[14] found in their study that vitamin D deficiency was significantly more common among the smokers (57%) than among nonsmokers (22%).

Conclusion

This study showed that vitamin D deficiency is prominent in patients with head and neck cancer before treatment than in controls. Because vitamin D deficiency may expose the patients to increased risk of therapy-related morbidity and poor outcome, it may constitute an inexpensive prophylactic and cost-effective option in the therapeutic armamentarium as a synergistic agent to traditional treatment options.

However, because there is no consensus on the appropriate serum vitamin D levels for global health, the doses to use for its supplementation, or the cutoffs for its deficiency, large controlled, prospective studies are needed on VD supplementation to clarify whether it has a role in the pathogenesis, prevention of, and treatment outcome in patients with head and neck cancer.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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