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 [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. Thus, it has been hypothesized that vitamin D may protect against cancer at multiple sites. Experimental study on mice showed

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 (<37.5nmol/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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that therapy with 1,25-dihydroxyvitamin vitamin D₃ [1,25(OH)₂D₃] 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)₂D₃ exerts its antitumor effects is still a myth [9,10].

Other studies showed that 1,25(OH)₂D₃ 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.

Serum levels of 25(OH)D₃ (vitamin D) were measured by enzyme-linked immunosorbent assay (ELISA) technique using CALBIOTECH ELISA kit for Human VD3 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 VD₃. Awareness Technology Inc. Star Fax 2100 (Westport, UK) was used as the ELISA reader.

Serum Ca and PO₄ 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 χ²-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) | 0.186a  |
| Male/female            | 20/10          | 36/14      | 0.614b  |
| Smoker/nonsmoker       | 24/6           | 37/13      | 0.542b  |

Data are presented as mean (SD) or ratio. a Unpaired t-test. b Pearson χ²-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 (%).

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 mmol/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),
respectively; $P$ value was 0.223, which reflects no statistical significant decrease in the serum PO4 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.

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<sup>a</sup> |
| Calcium level (mmol/l)   | 2.6 (2.300–2.80) | 2.4 (1.900–2.70) | 0.097<sup>a</sup> |
| Phosphate level (mmol/l) | 1.01 (0.89–1.30) | 1.15 (0.89–1.40) | 0.223<sup>a</sup> |

Data are presented as median (interquartile range).<sup>a</sup>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<sub>3</sub> 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<sup>a</sup> |
| 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<sup>a</sup> |
| 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<sup>a</sup> |

Data are presented as median (interquartile range).<sup>a</sup>Kruskal–Wallis test. HNC, head and neck cancer.

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<sub>3</sub> has clinical effectiveness in a hamster buccal pouch tumor model [3]. VD<sub>3</sub> 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
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 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 VD3 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 VD3 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 VD3 treatment.

| Dependent variable | Independent variables | \( B \) | SE | \( r_{\text{partial}} \) | \( t \) | \( P \) 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.
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 PO4 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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**Conflicts of interest**

There are no conflicts of interest.
REFERENCES

1 National Cancer Institute. Surveillance epidemiology and end results. Available at: http://seer.cancer.gov/statfacts/html/oralcav.html. [Last accessed on 21 May 2013].

2 Buttigliero C, Monagheddu C, Petroni P, Saini A, Dogliotti L, Ciccone G, Bernut A. Prognostic role of vitamin D status and efficacy of vitamin D supplementation in cancer patients: a systematic review. Oncologist 2011; 16:1215–1227.

3 Meier JD, Enepekides DJ, Poirier B, Bradley CA, Albala JS, Farwell DG. Treatment with 1α,25-dihydroxyvitamin D3 (vitamin D3) to inhibit carcinogenesis in the hamster buccal pouch model. Arch Otolaryngol 2007; 133:1149–1152.

4 Helzlsouer KJ. Overview of the cohort consortium vitamin D pooling project of rarer cancers. Am J Epidemiol 2010; 172:4–9.

5 Wiers KM, Lathers DM, Wright MA, Young MR. Vitamin D3 treatment to diminish the levels of immune suppressive CD34+ cells increases the effectiveness of adoptive immunotherapy. J Immunother 2000; 23:115–124.

6 Ma Y, Zhang P, Wang F, Yang J, Liu Z, Qin H. Association between vitamin D and risk of colorectal cancer: a systematic review of prospective studies. J Clin Oncol 2011; 29:3775–3782.

7 Bolland MJ, Greer GA, Gamble GD, Reid IR. Calcium and vitamin D supplements and health outcomes: a reanalysis of the Women’s Health Initiative (WHI) limited-access data set. Am J Clin Nutr 2011; 94:1144–1149.

8 Vielting A, Hein R, Abbas S, Schneeweiss A, Flesch-Janys D, Chang-Clauw J. Serum 25-hydroxyvitamin D and postmenopausal breast cancer survival: a prospective patient cohort study. Breast Cancer Res 2011; 13: R74.

9 Chiang KC, Yeh CN, Chen HY, Lee JM, Juang HH, Chen MF, et al. 19-Nor-2α-(3-hydroxypropyl)-1α,25-dihydroxyvitamin D3 (MART-10) is a potent cell growth regulator with enhanced chemotherapeutic potency in liver cancer cells. Steroids 2011; 76:1513–1519.

10 Kovalenko PL, Zhang Z, Yu JG, Li Y, Clinton SK, Fleet JC. Dietary vitamin D and vitamin D receptor level modulate epithelial cell proliferation and apoptosis in the prostate. Cancer Prev Res 2011; 4:1617–1725.

11 Kulbersh JS, Day TA, Gillespie MB, Young MR. 1α,25-Dihydroxyvitamin D3 to skew intratumoral levels of immune inhibitor CD34+ progenitor cells into dendritic cells. Otolaryngol Head Neck Surg 2009; 140:235–240.

12 Lin R, Nagai Y, Sladek R, Bastian Y, Ho J, Petrecca K, et al. Expression profiling in squamous carcinoma cells reveals pleiotropic effects of vitamin D analog EB1089 signaling on cell proliferation, differentiation, and immune system regulation. Mol Endocrinol 2002; 16:1243–1256.

13 Fleet JC, DeSmet M, Johnson R, Li Y. Vitamin D and cancer: a review of molecular mechanisms. Biochem J 2012; 441:51–71.

14 Oreil-Kotikangas H, Schwab U, Österlund P, Mäkitie O, Mäkitie AA. High prevalence of vitamin D insufficiency in patients with head and neck cancer at diagnosis. Head Neck 2012; 34:1450–1457.

15 Lack of association between serum levels of 25-hydroxyvitamin D and the incidence and mortality in men. J Natl Cancer Inst 2006; 98:451–462.

16 Nishii Y, Ogata E, Ikeda K. Antitumor effect of 22-oxa-calcitriol, a nonacemic analogue of calcitriol, in athymic mice implanted with human breast carcinoma and its synergism with tamoxifen. Cancer Res 1993; 53:2534–2539.

17 Dahlof K, Danzey J, Astrup A, Skovgaard T, Hamberg KJ, Lofos FJ, et al. A phase II study of the vitamin D analogue seocalcitriol in patients with inoperable hepatocellular carcinoma. Br J Cancer 2003; 89:252–257.

18 Giovannucci E, Liu Y, Rimm EB, Hollos BW, Fuchs CS, Stampfer MJ, Willett WC. Prospective study of predictors of vitamin D status and cancer incidence and mortality in men. J Natl Cancer Inst 2006; 98:451–459.

19 Cui Y, Rohan TE. Vitamin D, calcium, and breast cancer risk: a review. Cancer Epidemiol Biomarkers Prev 2006; 15:1427–1437.

20 Schwartz GG. Vitamin D and the epidemiology of prostate cancer. Semin Dial 2005; 18:276–289.

21 Schwartz GG, Skinner HG. Vitamin D status and cancer: new insights. Curr Opin Clin Nutr Metab Care 2007; 106–11.

22 Holick MF. Vitamin D The underappreciated D-lightful hormone that is important for skeletal and cellular health. Curr Opin Endocrinol Diabetes 2002; 9:867–96.

23 Holick MF. Vitamin D: photobiology, metabolism, mechanism of action, and clinical applications. In: Favus MJ, eds. Primer on the metabolic bone diseases and disorders of mineral metabolism 5th ed.Washington, DC: American Society for Bone and Mineral Research; 2003;129–137.

24 Gregori S, Giaratana N, Simiolo S, Usovskik M, Adorini L A, 25-dihydroxyvitamin D3 analog enhances regulatory T-cells and arrests autoimmune diabetes in NOD mice. Diabetes 2002; 51:1367–1374.

25 Young MR, Day TA. Immune regulatory activity of vitamin D3 in head and neck cancer. Cancers (Basel) 2013; 5:1072–1085.

26 Li J, Jin D, Fu S, Mei G, Zhou J, Lei L, et al. Insulin-like growth factor binding protein-3 modulates osteoblast differentiation via interaction with vitamin D receptor. Biochim Biophys Acta 2013; 436:632–637.

27 Lathers DM, Lubbers E, Wright MA, Young MR. Dendritic cell differentiation pathways of CD34+ cells from the peripheral blood of head and neck cancer patients. J Leukoc Biol 1999; 65:623–628.

28 Ginde AA, Liu MC, Camargo CA Jr. Demographic differences and trends of vitamin D insufficiency in the US population, 1988–2004. Arch Int Med 2009; 169:626–632.

29 Godar DE, Pope SJ, Grant WB, Holick MF. Solar UV doses of adult Americans and vitamin D3 production. Dermatolendocrinol 2011; 3:243–250.

30 Walsh JE, Clark AM, Day TA, Gillespie MB, Young MR. Use of 1α,25-dihydroxyvitamin D3 treatment to stimulate immune infiltration into head and neck squamous carcinoma. Human Immunol 2010; 71:659–666.

31 Moreno J, Krishnan AV, Peehl DM, Feldman D. Mechanisms of vitamin D-mediated growth inhibition in prostate cancer cells: inhibition of the progestational pathway. Anticancer Res 2006; 26:2525–2530.

32 Thill M, Fischer D, Hoelten F, Kelling D, Dittmer C, Landt S, et al. Prostaglandin metabolising enzymes and PGE2 are inversely correlated with vitamin D receptor and 25(OH)D2/3 in breast cancer. Anticancer Res 2010; 30:1673–1679.

33 Thill M, Fischer D, Kelling F, Hoelten F, Dittmer C, Horнемann A, et al. Expression of vitamin D receptor (VDR), cyclooxygenase-2 (COX-2) and 15-hydroxyprostaglandin dehydrogenase (15-PGDH) in benign and malignant ovarian tissue and 25-hydroxycholecalciferol (25(OH)2D3) and prostaglandin E2 (PGE2) serum level in ovarian cancer patients. J Steroid Biochem Mol Biol 2010; 121:387–390.

34 Dong J, Wong SL, Lau CW, Liu J, Wang YX, He ZD, et al. Calcitrol restores renal renovascular function in estrogen-deficient rats through downregulation of cyclooxygenase-2 and the thromboxane-prostanoid receptor. Kidney Int 2013; 84:54–63.

35 Rinkonen J, Wolff H, Paavonen T. Expression of cyclooxygenase-2 in human tongue carcinoma and its precursor lesions. Virchows Arch 2002; 440:594–597.

36 Mulligan JK, Day TA, Gillespie MB, Rosenzweig SA, Young MR. Secretion of vascular endothelial growth factor by oral squamous cell carcinoma cells skews endothelial cells to suppress T-cell functions. Human Immunol 2009; 70:375–382.

37 Wanebo HJ, Riley T, Karz D, Pace RC, Johns ME, Cantrell RW. Indomethacin sensitive suppressor-cell activity in head and neck cancer patients. The role of the adherent mononuclear cell. Cancer 1988; 61:462–474.

38 Dovedi SJ, Kirby JA, Davies BR, Leung H, Kelly JD. Celecoxib has potent antitumour effects as a single agent and in combination with BCG immunotherapy in a model of urthelial cell carcinoma. Eur Urol 2008; 54:621–630.

39 Young MR, Newby M. Differential induction of macrophage prostaglandin B2 secretion and suppressor activity by Lewis lung carcinoma variants. In: Garaci E, Santoro MG, eds. Prostaglandins and cancer Berlin, Heidelberg, Germany: Springer-Verlag; 1987:273–276.

40 Gillespie MB, Moody MW, Lee FS, Poole LJ, Hornig JD, Lathers D, et al. Head and neck cancer recurrence and mortality in nonselective cyclooxygenase inhibitor users. Arch Otolaryngol 2007; 133:28–31.
44 Lang S, Lauffer L, Clausen C, Lohr I, Schmitt B, Holzel D, et al. Impaired monocyte function in cancer patients: restoration with a cyclooxygenase-2 inhibitor. FASEB J 2003; 17:286–288.

45 Zhou W, Heist RS, Liu G, Asomaning K, Neuberg DS, Hollis BW, et al. Circulating 25-hydroxyvitamin D levels predict survival in early-stage non-small-cell lung cancer patients. J Clin Oncol 2007; 25:479–485.

46 Smolders J, Thewissen M, Damoiseaux J. Control of T cell activation by vitamin D. Nat Immunol 2011; 12:3–4 3 author reply.

47 Need AG, Nordin BE. Misconceptions - vitamin D insufficiency causes malabsorption of calcium. Bone 2008; 42:1021–1024.

48 Weaver CM, McCabe LD, McCabe GP, Braun M, Martin BR, DiMeglio LA, Peacock M. Vitamin D status and calcium metabolism in adolescent black and white girls on a range of controlled calcium intakes. J Clin Endocrinol Metab 2008; 93:3907–3914.

49 Abrams SA, Griffin LJ, Hawthorne KM, Gunn SK, Gundberg CM, Carpenter TO. Relationships among vitamin D levels, parathyroid hormone, and calcium absorption in young adolescents. J Clin Endocrinol Metab 2005; 90:5576–5581.

50 Aloia JF, Chen DG, Yeh JK, Chen H. Serum vitamin D metabolites and intestinal calcium absorption efficiency in women. Am J Clin Nutr 2010; 92:835–840.

51 Chung M, Balk EM, Brendel M, Ip S, Lau J, Lee J, et al. Vitamin D and calcium: a systematic review of health outcomes. Evidence Report No. 183. (Prepared by the Tufts Evidence-based Practice Center under Contract No. HHSA 290-2007-10055-I.). Rockville, MD Agency for Healthcare Research and Quality 2009.

52 Brocq S, Jørgensen BN, Madsen OR, Jensen LB, Sørensen OH. Relationships between bone mineral density, serum vitamin D metabolites and calcium: phosphorus intake in healthy perimenopausal women. J Intern Med 1999; 10:509–516.

53 Aphan T, Bugdayci G, Alcelik A, Aktas G. Serum levels of vitamin D among patients with nasal polyps in Bolu, Turkey. J Aller Ther 2011; SS1.

54 Melamed ML, Michos ED, Post W, Astor B. 25-hydroxyvitamin D levels and the risk of mortality in the general population. Arch Intern Med 2008; 168:1628–1637.

55 MacLaughlin J, Holick MF. Aging decreases the capacity of human skin to produce vitamin D3. J Clin Invest 1985; 76:1536–1538.

56 Ebeling PR, Sandgren ME, DiMagno EP, Lane AW, DeLuca HF, Riggs BL. Evidence of an age-related decrease in intestinal responsiveness to vitamin D: relationship between serum 1,25-dihydroxyvitamin D3 and intestinal vitamin D receptor concentrations in normal women. J Clin Endocrinol Metab 1992; 75:176–182.