Oral cancer risk and vitamin D status, intake and supplementation: A review

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Abstract
Introduction
Vitamin D sufficiency is associated with a variety of human health benefits, while vitamin D deficiency has been identified as a potential risk factor for several health-related issues, including oral cancer. The goal of this critical review was to assess the research, epidemiologic evidence and mechanisms through which vitamin D may influence oral cancer risk or progression.

Discussion
Recent evidence now suggests that vitamin D exhibits several different effects on normal and cancerous cells, including up-regulation of anti-proliferation and pro-apoptotic factors, as well as inhibition of cell cycle promoters and growth factor signalling pathways, such as Wnt and mitogen-activated protein kinase (MAPK). Some studies, however, have demonstrated inconclusive results, which may be complicated by inadequate study design to account for baseline vitamin D status or deficiencies, and also by tumour-specific up-regulation of the vitamin D catabolism enzyme, cytochrome p450 24 (CYP24), or mutations in the vitamin D receptor (VDR), which have been observed in some oral cancers.

Conclusion
This comprehensive analysis of research regarding vitamin D status, intake and metabolism suggests oral cancer risk may, in fact, be more interconnected than previously acknowledged. Furthermore, more in-depth analysis of VDR and CYP24 expression, along with baseline vitamin D status, may elucidate some of the underlying mechanisms of oral cancer responsiveness, which may be useful to oral oncologists, oral health care providers and oral epidemiologists as they strive to improve patient health and outcomes.

Introduction
Oral cancer
Although the rates of oral cancer incidence and mortality have declined in the United States and other industrialized countries over recent decades, concomitant increases have been observed in other nations and worldwide, in general. As workplace participation and social mobility have increased along with disposable incomes in developing economies, the availability of tobacco and alcohol products has been associated with increasing rates of oral cancers. Studies of the primary risk factors for the development of oral cancers in the United States have found that tobacco use and, to a lesser extent, alcohol consumption, when combined, may be responsible for as much as 80% of this cancer risk.

However, these studies have also uncovered differing incidence and mortality rates among demographic subgroups within the population, including stark differences by age, sharp increases observed among females and much higher rates observed among minorities. An additional important risk factor for oral and pharyngeal cancers (OPCs) is oral infection with the human papilloma virus (HPV). Oral HPV infection may be disproportionately associated with specific demographic subgroups, such as men and some minority subgroups, which may underlie some of the divergent geospatial and geographic OPC trends observed.

Dietary influences
Although the majority of OPC risk in developed countries may be attributable to tobacco and alcohol consumption or HPV infection, several studies have recently demonstrated that opposing, health-protective effects and reduced incidence of OPC may be associated with intake of specific dietary components such as coffee, fibre, folic acid and the vitamins A, C, D and E. In fact, recent meta-analyses have demonstrated consistent, inverse associations between the intake of specific dietary components, such as folate, and the risk, development and progression of OPC. In addition, dietary limitations or restrictions and micronutrient deficiencies have been shown to increase OPC risk. It is difficult (and often problematic) to distinguish the effects of specific micronutrients or constituents in dietary intake studies from those of the foods that contain them. This may explain why researchers have concluded that dietary intake (but not specific vitamins or micronutrients) accounts for as much as 20–25% of the variability in OPC risk.
Vitamin D

Large-scale population studies have revealed that low serum vitamin D levels are associated with significant increases in cancer risk\textsuperscript{15,20}. More specifically, epidemiologic and case-controlled studies have now demonstrated that low vitamin D levels are strongly associated with OPC risk\textsuperscript{21,22}. In fact, more recent evidence now demonstrates that OPC patients are more likely to harbour vitamin D receptor (VDR) gene polymorphisms, mutations or deletions\textsuperscript{23–25}. Conversely, those with specific VDR mutations were at higher risk for developing OPC than those with normal genotypes\textsuperscript{26–28}.

Vitamin D, precursors are produced in the skin upon exposure to sunlight, but also may be ingested from dietary sources, such as animal food products, while vitamin D\textsubscript{3} may be obtained from plant-based products and synthetically manufactured dietary additives or nutritional supplements\textsuperscript{29,30}. No significant differences between the metabolism of the major circulating forms, vitamin D\textsubscript{2} \([25(\text{OH})\text{D}_{2}]\) and vitamin D\textsubscript{3} \([25(\text{OH})\text{D}_{3}]\) have been noted, with vitamin D precursors either from sun exposure or from diet hydroxylated in the liver via the p450 27A system to 25-hydroxyvitamin D \([25(\text{OH})\text{D}]\)\textsuperscript{31}. The circulating concentration of 25(\text{OH})D ranges between 20 and 150 nmol/l (9 and 60 ng/ml), which has a normal serum half-life of about 3 weeks, with vitamin D intoxication described at concentrations above 375 nmol/l (150 ng/ml)\textsuperscript{32}.

Although the primary site for vitamin D metabolism is hydroxylation in the hepatic p450 27A system, the kidney, bones and parathyroid gland are major sites of additional processing of 25(OH)D to the active form, 1,25(OH)\textsubscript{2}D, which is between 38 and 144 pmol/l (16 and 60 pg/ml) that has a circulation half-life between 4 and 6 hours\textsuperscript{30,31}. Vitamin D doses may also be stated in international units (IU), which adjusts for biological activity or effect with one IU of vitamin D defined as the activity of 0.025 \(\mu g\) of 1,25(OH)\textsubscript{2}D\textsubscript{3}\textsuperscript{29}. The simplest way to understand and compare the varying studies of vitamin D may be to apply the conversion of IU to grams of 1,25(OH)\textsubscript{2}D\textsubscript{3} 40 IU equals 1 \(\mu g\) in dietary sources, and to apply the conversion of clinical serum levels to \textit{in vitro} concentrations, 2.5 nmol/l equals 1 ng/ml\textsuperscript{30}.

Although vitamin D is routinely supplemented into dietary food staples of developed countries, the primary determinant of vitamin D status for the developing world is sun exposure, since vitamin D production in the skin is proportional to ultraviolet (UV) light exposure\textsuperscript{32}. For instance, although an eight ounce glass of milk in the United States is fortified to contain 100 IU of vitamin D, exposure of the skin to enough UV-B radiation to cause a slight pinkness in Caucasian skin produces the equivalent to an oral dose of 20,000 IU of vitamin D\textsuperscript{30,31}. These findings may explain the epidemiologic observations that increased sun exposure in certain geographic regions and among certain populations was associated with reduced cancer mortality and risk at all tumour sites. Data from more than 100 countries have demonstrated strong, inverse correlations between solar UVB exposure for 15 types of cancer and significant (although less robust) effects observed among nine other cancers, including those of the larynx and oral cavity\textsuperscript{19–22,32,33}.

Based upon this information, the primary objective of this study is to provide a critical review of not only the research and epidemiologic evidence but also the mechanisms through which vitamin D may influence OPC risk and progression.

Discussion

The authors have referenced some of their own studies in this review. These referenced studies have been conducted in accordance with the Declaration of Helsinki (1964), and the protocols of these studies have been approved by the relevant ethics committees related to the institution in which they were performed. All human subjects, in these referenced studies, gave informed consent to participate in these studies.

The primary mechanism of vitamin D action is mediated through binding of either 1,25(OH)\textsubscript{2}D (active form) or 25(OH)D (less active form) to the VDR, which is a member of the nuclear receptor superfamily of steroid and thyroid hormones with gene-regulatory and consequent anti-proliferative properties\textsuperscript{30,34}. Binding of 1,25(OH)\textsubscript{2}D to the VDR (either in the cell nucleus or in the cytoplasm) promotes association of the VDR-1,25(OH)\textsubscript{2}D complex with the retinoid X receptor (RXR)\textsuperscript{21,30}. The 1,25(OH)\textsubscript{2}D2D-VDR-RXR complex binds to vitamin D-response elements in DNA which operate to initiate gene transcription. Activation of the VDR by 1,25(OH)\textsubscript{2}D can restore or enhance proapoptotic effects in different cancer cells through transcriptional activation of bax and p-celapain, two effective proapoptotic proteins\textsuperscript{35,36}. VDR-vitamin D activation also been demonstrated to increase mRNA expression of transforming growth factor, a potent anti-proliferative cytokine in normal and early stage cancer cells; superoxide dismutase, which may reduce oxidative stress-induced DNA damage and loss to DNA repair mechanisms that contribute to carcinogenesis and inflammatory cytokine production; as well as cyclin-dependent kinase (CDK) inhibitor p21, RBL2, RBLP6 and forkhead box O (FOXO) tumour suppressors that function to counteract MAPK-mediated phosphorylation and growth\textsuperscript{35,37–39}.

VDR activation may also facilitate transcriptional repression of Bcl-2...
and telomerase (pro-survival proteins), as well as CDK1 mRNA, which encodes a required protein for cell-cycle progression. Suppression of vascular endothelial growth factor; responsible for angiogenesis, as well as the pro-inflammatory cyclooxygenase-2, was also observed. In addition, 1,25(OH)D may disrupt the function of β-catenin, the terminal mediator of Wnt signalling, which activates transcription of genes whose protein products (c-Myc and cyclin D1) control cell proliferation, as well as insulin-like growth factor–stimulated tumour growth.

Despite these documented anti-cancer properties, some recent studies have demonstrated possible adverse effects of elevated 25(OH)D concentrations on cancer risk in prostate, breast, pancreas and oesophageal cancers, suggesting that these effects may depend on dose, timing and duration of exposure, as well as tissue-specific, lifestyle and genetic factors. Although J- or U-shaped risk curves have been proposed to describe the noted associations in these studies, confounding factors present in the original studies are likely responsible for these findings. For example, outcomes of intervention trials of supplemental vitamin D were inconclusive due to the lack of baseline vitamin D status reports of trial participants and consequent dose adequacy estimates. This may suggest that studies focused on the dose administered, rather than their effect on alleviating deficiency, achieving adequacy or adding to pre-trial adequate serum levels, would have significantly affected the response curves and complicated the interpretation of trial outcomes.

One additional concern with these studies is a lack of control for confounding variables. Many cancers, including OPC, may exert effects on vitamin D metabolism by changing the availability of, or affecting the ability to bind to, the VDR. For example, there is some evidence that specific cancers exhibit reduced VDR expression. There is also evidence that Ras activation, common in many OPC cancers, may impair vitamin D-mediated transcription activity, while cytochrome p450 24 (CYP 24), the enzyme responsible for degradation of vitamin D metabolites, may be functionally active and up-regulated in many tumours.

These tissue-specific characteristics may also explain the varying results obtained in experimental in vitro studies of 1,25(OH)D on gene expression in several squamous cell carcinoma (SCC) head and neck cell lines (SCC4, SCC9, SCC15 and SCC25), which demonstrated differing sensitivities among the cell lines ranging from complete cell-cycle arrest at G1/G0 for SCC25 to only 50% inhibition of growth for SCC9. Screening of more than 4,500 target genes yielded 38 up-regulated (at least 1.5 fold) target genes in SCC25 cells, including cell adhesion proteins, growth factors, cytoskeleton proteins, protein kinases, other intracellular signalling molecules and transcription factors previously implicated in control of cell-cycle growth and arrest. Although no change in expression of p27 or p53 mRNA levels was observed, and only a modest induction of p21 transcription was noted, another study using microarray technology to profile target gene regulation in SCC25 head and neck SCCs revealed 89 up-regulated and 63 down-regulated genes. The gene coding for cytochrome p450 24 [the protein that degrades 1,25(OH)D3] exhibited the highest up-regulation of 196-fold. This confirmed the findings of another study that found CYP24 mutations lowered oral cancer risk compared with wild type, after adjusting for age, gender, alcohol consumption and smoking status.

Conclusion

The primary goal of this critical review was to explore the research and evidence regarding the mechanisms through which vitamin D might modulate the risk or progression of OPC. Although many clinical studies have suggested vitamin D status, intake and supplementation may have a significant influence on oral cancer risk, progression and mortality, growing epidemiologic evidence now suggests that dietary supplementation may not provide levels similar to UVB exposure. As work environments in the developing world are increasing indoors and the obesity crisis further limits physical activity and outdoor exposure, the impact and influence of vitamin D intake may become increasingly critical. In addition, although many studies have demonstrated the anti-tumour effects of vitamin D both in vitro and in vivo, new evidence suggests these effects are modulated by other factors, including tissue and tumour regulation of CYP24. These data combined suggest that more research is needed to examine the in vitro effects of vitamin D on OCP which include analyses of CYP27 and CYP24 activity and VDR expression, while clinical and in vivo studies should more closely examine the relationship between baseline vitamin D status and alleviating deficiency, achieving adequacy. This information may be useful to oral oncologists, oral health care providers and oral epidemiologists as they strive to improve patient health and outcomes.

Abbreviations list

CDK, cyclin-dependent kinase; FOXO, forkhead box O; HPV, human papillomavirus; IU, international unit; MAPK, mitogen-activated protein kinase; OPC, oral and pharyngeal cancer; RXR, retinoid X receptor; SCC, squamous cell carcinoma; UV, ultraviolet; VDR, vitamin D receptor

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