

Vitamin D: relevance in dental practice

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Summary

Objective. The potential role of VDR gene variations in modulating periodontal susceptibility have been a subject of scientific investigations. The aim of this paper is to perform a literature review of the potential correlation between Vitamin D Receptor (VDR) gene polymorphisms and periodontal disease.

Materials and methods. A PubMed literature search was made using “vitamin d receptor polymorphisms periodontal disease” as keys words. Only clinical studies in “Humans” as species and “English” as language were considered. Titles and abstracts of all identified records were examined to determine if the candidate articles contained sufficient information on the association of the VDR polymorphisms and the risk of development periodontal disease.

Conclusions. Vitamin D may affect the risk of developing periodontal disease via an effect on bone mineral density or via immunomodulatory effects. There are scientific evidences about the correlation between some VDR polymorphisms, periodontitis and bone metabolism. The use of new simple and economics diagnostic techniques of genetic screening, allows to the dental specialists to identify periodontal patients with possible decreased bone mineral density. The complete acquisition of awareness by dentists of the strong relationship between skeletal bone density with periodontal health and osteointegrated implant success, could open a new therapeutic approach for periodontists with an important role in the early detection of osteoporosis and a better patient compliance of the periodontal therapy.

KEY WORDS: periodontitis; vitamin D receptor polymorphisms; vitamin D; periodontal disease; genetic susceptibility periodontitis.

Introduction

Vitamin D is a well known regulator in musculoskeletal health through the mediation of calcium absorption and mineral homeostasis. Evidence has demonstrated that vitamin D deficiency may place subjects at risk not only for low mineral bone density but also in other metabolic pathways such as those involved in immune response, chronic inflammatory diseases and cancer (1). However, there are no studies demonstrating the role that vitamin D deficiency can get in the etiopathogenesis of oral disease such as periodontitis. Moreover, dentists have not yet well understood the importance of a good bone metabolism for a successful dental practice in bone regeneration procedures and osseointegration of implants. An insufficient bone base which cannot guarantee the necessary stability of the implants placed in it, has been one of the biggest problems of implantology in the past.

Periodontitis is a multifactorial inflammatory disease of the periodontium that represents an increasing serious health problem in the worldwide population so that the World Health Organization has included this pathology among its primary prevention programs (2). The relationship between periodontitis and systemic diseases has increasingly recognized due to the fact that the periodontal pathogens might affect distant sites and organs, and thus have an effects on overall health. A large number of epidemiological studies have recently linked poor oral health with cardiovascular diseases, diabetes, low birth weight, preterm births, rheumatoid arthritis etc. (3-6). The microbiological shift of the microbial communities found in gingival sulcus from predominantly aerobic, gram-positive and commensal oral bacterial species to predominantly gram-negative and anaerobic bacterial species, is essential to the development of periodontitis. However, the individual susceptibility to periodontal disease is strongly influenced by host immune response. Scientific evidences support the concept of periodontal infectogenomics which highlights how the host genetic profile modulates the composition of the subgingival microbiota, affecting persistence of pathogenic bacteria and therefore increasing the risk for the development of periodontitis. These studies emphasize the importance of the assessment of genetic profile in periodontal patients, in order to develop a personalized periodontal treatment plan (7). It is precisely because of its effects on bone metabolism, that Vitamin D could have a central role in dental practice. This review focuses on scientific evidences for a potential correlation between Vitamin D Receptor (VDR) polymorphisms and periodontal disease.

Vitamin D physiology

Vitamin D₃ (cholecalciferol) is a secosteroid produced in the skin by a photochemical reaction of 7-dehydrocholesterol. This initial vitamin D compound is inactive and it is then hydroxylated at the 25-position in the liver by the 25-hydroxylase hepatic, to form 25 OH vitamin D₃ (25-hydroxycholecalciferol).

ciferol), an inactive compound as well, but the most reliable measurement of an individual's vitamin D status. The 25OH vitamin D₃ is further hydroxylated in the kidney to the active compound 1,25 dihydroxyvitamin D₃ (1,25(OH)₂D₃ or calcitriol). 1,25(OH)₂D₃ acts on the intestine, where it stimulates calcium re-absorption, and upon bone, where it promotes osteoblast differentiation and matrix calcification (8).

Vitamin D receptor polymorphisms

The biologically most active metabolite of vitamin D 1,25(OH)₂D₃ (calcitriol) acts by binding an intracellular receptor Vitamin D Receptor (VDR), which is present in a wide variety of tissues. VDR belongs to a superfamily of transacting transcriptional regulatory factor, such as for example the steroid and thyroid hormone receptors. The gene encoding the VDR is located on the chromosome 12 (12q13.11) and encompasses two promoter regions, eight protein-coding exons and six untranslated exons (9). The major steps involved in the control of gene transcription by VDR include ligand binding, heterodimerization with retinoid X receptor, binding of the heterodimer to vitamin D response elements, and recruitment of other nuclear proteins into the transcriptional pre-initiation complex. The ensemble of protein-protein-DNA interactions promotes transcriptional initiation of a battery of target genes, leading to the pleiotropic effects of the 1,25(OH)₂D₃ hormone (10). Researchers have identified over 200 genes directly influenced by vitamin D and 2776 binding sites for the vitamin D receptor along the length of the genome. These were unusually concentrated near a number of genes associated with susceptibility to autoimmune conditions such as Crohn's disease, lupus and rheumatoid arthritis (11).

It can therefore be supposed that VDR gene polymorphisms and alterations on the VDR mediated signaling pathways could lead to different important cellular effects on gene activation such as enhanced/reduced transcription, calcium metabolism, cell proliferation and immunological response.

VDR gene contains 4 polymorphic regions, three of which located at the 3'-end and could be detected by restriction fragment length polymorphisms (RFLPs) using the restriction enzymes *BsmI*, *Apal* (intron 8) and *TaqI* (exon 9), while the fourth is detected by restriction enzyme *FokI* in the start codon (12, 13).

FokI polymorphism creates a start codon in exon 2 resulting in an alternative start site due to thymine (T) to cytosine (C) substitution in the first translation initiation codon (ATG to ACG), which generates long and short variants of *VDR*. Individuals with the T allele (designated f) initiate translation at the first ATG site, giving rise to a full length *VDR* protein (427 amino acids). Conversely, individuals with the C allele (designated F) initiate translation at the second ATG site instead of the first, resulting in a truncated protein with three amino acids less. This is the only known *VDR* polymorphism resulting in two different *VDR* protein products (14).

The *Apal* and the *BsmI* polymorphisms of the *VDR* gene are considered to be silent single nucleotide polymorphisms (SNPs). These polymorphisms (G/T and A/G respectively) do not change the amino acid sequence, the amount or function of the *VDR* encoded protein. However, they may affect gene expression through regulation of mRNA stability (15, 16).

The *VDR* *TaqI* (-1056) is characterized by a single base transition (T < C) leading to a synonymous change at codon

352 in exon 9 of the *VDR* gene that creates a *TaqI* restriction site. The resulting alleles are called "t" (*TaqI* site present) and "T" (*TaqI* site absent). The allele "t" correlates with increased transcriptional activity, mRNA stability, and high serum level of 1,25-D₃ (17, 18). However, the mechanisms by which the *BsmI*, *TaqI*, and *Apal* polymorphisms affect *VDR* function are still unclear.

The *CDX2* polymorphism is located in the promoter region of the *VDR* gene in exon 1, more exactly in a binding site for the *Cdx2*, an intestinal specific transcription factor that has been shown to activate *VDR* gene transcription in the intestinal tract (19). This polymorphism consists in a G to A substitution and results in a more active and strongly binding of *Cdx2* transcription factor in case of allele A, with greater transcriptional activity respect to the G allele. As consequence, the A allele may induce an higher *VDR* expression in the intestine and therefore an increased bone mineral density (BMD) through a better intestinal absorption of calcium (16, 20, 21).

Several studies have shown the presence of linkage disequilibrium across the *VDR* gene. Linkage disequilibrium can be defined as the co-occurrence of alleles of adjacent polymorphisms with each other, so the presence of a polymorphism allows us to predict what it is linked to. With reference to *VDR* gene there is concordance of a strong linkage disequilibrium between *BsmI*, *TaqI* and *Apal* while *FokI* polymorphisms don't appear to be in linkage disequilibrium with any other *VDR* polymorphisms and can be considered as an independent marker in the *VDR* gene (16, 22). Even though the great number of studies that have emphasized the association with *VDR* polymorphisms and various diseases, the real function of these *VDR* alleles on gene transcription, are not yet fully well understood, thus suggesting that these polymorphisms may occur in linkage disequilibrium with other functional polymorphisms in the *VDR* gene. Association studies have underlined the correlations of these *VDR* polymorphisms with autoimmune diseases such as rheumatoid arthritis, inflammatory bowel disease, multiple sclerosis, type I diabetes mellitus, but also cancer, increased susceptibility to microbial infection (periodontal disease) and obviously diseases related to disorders of bone metabolism (osteoporosis). It should be noted, as in all studies of genetic variation, that the results produced in literature could be affected by ethnic variations in the occurrence of *VDR* polymorphisms.

VDR polymorphisms and periodontal disease: a review of literature

A PubMed (National Center for Biotechnology, National Library of Medicine) literature search was made using "vitamin d receptor polymorphisms periodontal disease" as key words with additional activated filters "Humans" as species and "English" as language. Titles and abstracts of all identified records were examined to determine if the candidate articles contained sufficient information on the association of the *VDR* polymorphisms and the risk of development periodontal disease.

TaqI

Most of the articles focused on the analysis of *VDR* *TaqI* polymorphism in order to evaluate the potential association of this *VDR* gene polymorphism to an increased individual

susceptibility in developing periodontitis associated with alveolar bone loss. In particular, the TT genotype and the presence of T allele are associated with chronic periodontal disease in Japanese, Chinese and Caucasian subjects (23-27). This data is also in accordance with our previous study which showed a strong correlation between TT genotype of Italian subjects and chronic periodontitis (OR 3.59) but also with aggressive periodontal disease (28). In a cross-sectional study, Borges and co-authors examine the relationship between TaqI polymorphism and subgingival microbiota in Brazilian adults with chronic periodontitis, showing an association of Tt genotype with the disease without observing any association between genotype and bacterial component (29). The VDR TT genotype has been correlated with lower serum levels of Vitamin D₃ (17) and with a lower bone mineral density value in postmenopausal woman predisposed to a major risk of osteoporosis (30). Low serum levels of Vitamin D₃ due to reduced activation of the provitamin D by UV radiation, to VDR polymorphisms or to nutritional factors, are associated with increased circulating-reactive protein (31), with cardiovascular disease (32) and with autoimmune diseases such as inflammatory bowel disease, rheumatoid arthritis and multiple sclerosis (33). Furthermore, Vitamin D deficiency induces a decreased bone mineral density at skeletal level including maxilla and mandible, with an increased alveolar porosity and more rapid alveolar bone re-absorption following invasion by periodontal pathogens. These evidences may explain a higher susceptibility to periodontitis of patients showing VDR TT genotype, hypothesizing a more difficult host response to periodontopathogenic bacteria and to a marked bone loss. On the contrary, the studies of Hennig and Sun found an association between the carriage of the less-frequent VDR t allele with an increased risk of developing early-onset periodontal disease in Caucasian and Chinese subjects respectively (34, 35)

Apal

By comparing periodontal disease progression and Apal VDR polymorphism in adult men, Inagaki et al. demonstrated an association between AA genotype and the highest rates of progression of alveolar bone loss and tooth loss compared to Aa or aa genotypes (36). Moreover, Naito et al. found that AA genotype correlates with an increased risk of severe chronic periodontitis (37) among Japanese males.

BsmI

Previous studies conducted on healthy subjects and periodontal patients in Japanese and Brazilian population, agree in rejecting the hypothesis of the correlation between the only BsmI polymorphism and periodontal disease since no statistical differences in distribution of BsmI VDR polymorphism are found between the two groups. Vice-versa, in combination with Fc-gammaRIIIB genotype, BsmI may be associated with generalized early-onset periodontitis or, as demonstrated by de Brito, when the allele B of BsmI polymorphism and the T allele of TaqI polymorphism were present, the TB haplotype seemed to increase susceptibility to chronic periodontitis (37-39). The findings of Gunes indicates that the Apal, BsmI and TaqI polymorphisms analyzed individually, did not show statistically significant differences in their frequencies in a Turkish population of healthy subjects and patients affected by chronic periodontal disease while, also in this case, the presence of allele T (TaqI) and B (BsmI) in the haplotype BT is over-represented among chronic periodontal cases (40).

FokI

Park et al. found a correlation between the ff genotype of the FokI VDR polymorphism, with a greater individual susceptibility to developing generalized aggressive periodontal disease. This data was confirmed by the study of Naito and could be explained by the fact that ff genotype seems to be responsible for an increased bone re-absorption and an enhanced inflammatory response (37, 41). On the contrary, the study of Li and co-authors highlights as the Chinese patients showing the F allele have an increased risk of developing generalized aggressive periodontitis (42).

CDX2

To our knowledge there have not been yet studies of correlation between CDX2 polymorphisms and periodontal disease.

Vitamin D and periodontal disease

1,25(OH)₂D₃ plays a role in maintaining oral health through its effects on bone and mineral metabolism and its anti-inflammatory and immunomodulatory properties (43). Since alveolar bone re-absorption is a major characteristic of periodontal disease, it is possible to speculate that mediators of bone metabolism like VDR and its genetic polymorphisms play a role in determining the individual susceptibility to developing periodontitis. For these reasons VDR gene could be considered an interesting candidate gene usable for screening in periodontal practice, singularly or in combination with other inflammatory markers, and vitamin D supplementation could represent a simple attractive way of regulating bone loss in periodontitis. Moreover, according to the Vitamin D Council, recent estimates indicate that over 50% of the global population risks Vitamin D deficiency across all age groups and such represents one of the most underestimated health problems in the world (44, 45). In addition to genetic factors, about 80% of Vitamin D levels in humans are prevalently influenced by exposure to sunlight and approximately 15% by dietary intake. Vitamin D status varies according to latitude, season, skin pigmentation, work occupation (outdoor or indoor), age (elderly may live mostly indoor) and dietary habits.

There is convincing evidence, that vitamin D insufficiency can be considered an universal risk factor for several multifactorial diseases as, for example, metabolic disorders, arterial hypertension, multiple sclerosis, autoimmune, colorectal and breast cancer, cardiovascular disease and osteoporosis. Osteoporosis may be a predisposing factor for alveolar bone loss in periodontal disease and vice versa, periodontitis could be an earliest sign of osteoporosis. Decreased bone density in the active sites of periodontitis may result in favouring a consequent accelerated progression of the host inflammatory response to the parodontopathogens, the elevation of systemic levels of cytokine that further increases the loss of bone density and risk of tooth loss (46). Cross-sectional studies showed evidence that mandibular bone mass was significantly correlated with skeletal bone mass (47-50). The bisphosphonates are first-line therapy for the treatment of most patients with osteoporosis, with proven efficacy to reduce fracture risk at the spine, hip and other non-vertebral skeletal sites. Further, bisphosphonates have been associated with a significant decrease in morbidity and increase in survival (51, 52). This treatment induces cell death in osteoclasts populations with a consequent reduction of

bone re-absorption in osteoporosis and loss of mineralization, but it is also associated with an increased risk of osteonecrosis of the jaw combined with dental surgery. The osteonecrotic lesions include erythematous chronic ulceration of the oral mucosa, with outcrops of underlying necrotic bone, often associated with the presence of suppurative phlogosis, jaw fractures and extra-oral fistulae (53, 54). Before beginning any surgical treatment, dentists must take an adequate action of treatment of endodontic and periodontal infective foci and evaluate the bacterial component with specific microbiological tests. It is also necessary to verify the serum levels of Vitamin D, in patients in treatment with bisphosphonates, before assuming eventual supplements. Patients must take along with Vitamin D supplements a preventive antibiotic treatment, one week before and one week after surgery. The intake of Vitamin D, along with the treatment with bisphosphonates, can avoid the occurrence of osteonecrosis and contributes to a reduction of the symptoms and of the infectious complications related to this pathology.

Conclusions

These findings can open a new therapeutic approach for periodontists. Dental practice, in fact could have an important role in the early detection of osteoporosis. With the advent of new simple and economics diagnostic techniques of genetic screening, it is possible for the dental specialist to identify periodontal patients with decreased bone mineral density and thus refer the patient to a evaluation of BMD by DEXA. Periodontal patients also diagnosed with osteoporosis/osteopenia should be clinically treated more aggressively to eliminate periodontal pathogens because of the additive risks. Eventually, the dentist could address the patient to an endocrinological specialist to evaluate pharmacological approaches such as ordinary Vitamin D supplements or more specific drugs such as bisphosphonates, calcitonin etc. and lifestyle intervention.

The complete acquisition of awareness by dentists of the strong relationship between skeletal bone density with periodontal health and osteointegrated implant success, also allows patients to better comply to the periodontal therapy, perceiving the periodontists or the dental team as active participants in improving and promoting the general health of patients.

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