Chapter

The Importance of Vitamin D for Periodontal Tissues

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Abstract

There are many causes of vitamin D deficiency, which determines pathogenesis of many diseases, including periodontal ones. Constant low uptake or deficiency of vitamin D results in progression of periodontal diseases and jaw bone metabolism - leads to change of bone mineral density, causes resorption in alveolar bone, tooth loss, changes of masticatory function and osteoporosis. The clinical studies strive to link vitamin D with gingivitis and periodontitis and prove its therapeutic and preventive role, because of vitamin D immunomodulatory, anti-inflammatory and antiproliferative effects for periodontal tissues and best treatment outcome. The purpose of this chapter is to analyze the importance of vitamin D on the pathogenesis of periodontal diseases, its role on regulation of the immune system and defense mechanism, influence on jawbone quality and on the correlation between vitamin D concentration in plasma and periodontal diseases.

Keywords: vitamin D, vitamin D deficiency, periodontal disease, jawbone, bone mineral density

1. Introduction

One billion people on the planet were diagnosed with vitamin D deficiency during the last decade [1]. Opinion, advocating important role of vitamin D and its deficiency in significant number of individuals, prevails within the society, a lot of information is available regarding its use, doses, sources, etc. However, each individual may have different vitamin D needs and the consumption of it should be monitored by testing individual serum levels of D_2 (of alimentary origin) and D_3 (synthesized in the skin), thus evaluating total level of vitamin D [2]. There are many causes of vitamin D deficiency, which is finally diagnosed if serum level of $25OHD_3$ is less than 20 ng/mL [3, 4]. This determines pathogenesis of many diseases, including periodontal ones, resulting in loss of masticatory function.

Periodontium (PT) consists of gingiva, periodontal ligament, cementum and alveolar bone. This is functional unit, maintaining homeostasis – the connection between tooth and gingiva makes up a unified whole, preventing penetration of microorganisms, chemical substances, capable to induce inflammation of periodontal tissues (PTs). The periodontal ligament and bone keep tooth inside the alveolar socket, distribute mechanical load of mastication. PT has its own blood supply, neural regulation and defensive mechanisms. Soft and mineralized dental plaque is initiative risk factor of periodontal diseases (PD) – bacterial biofilm and its adhesion to tooth surface induces response of PTs, but in general, diseases are caused by many predisposing factors. PD initially manifest as gingivitis, which, if untreated, spreads

deeper within PT: causing attachment loss, periodontal pocket formation, alveolar bone resorption and tooth loss over time [5, 6]. Increasing number of studies proves importance of vitamin D in prevention and management of oral diseases [7]. Clinical studies strive to link vitamin D with PD and prove its therapeutic and preventive role. Vitamin D is secosteroidal hormone, playing important role in the treatment of gingivitis and periodontitis because of its anti-inflammatory and antibacterial effect on PTs as well as its immunomodulatory, differentiating, anti-proliferative and regulative effect on autoimmune processes, cellular apoptosis and participation in bone metabolism [3, 7–10]. Disintegration and renewal processes of bone depend on metabolism, as constant interchange of mineral substances between bone and blood plasma, where an active form of vitamin D is circulating, takes place [11]. One of the main functions of vitamin D is maintaining of blood levels of calcium and phosphorus by regulating absorption of these substances inside the bowels and reabsorption in kidneys and by enhancing remodeling processes [12, 13]. Bone is like a living and continuously changing organ where resorption and regeneration, i.e. remodelation, take place [14]. For this reason, the old bone is not accumulating and adaptation to changing mechanical forces develops. Jawbone support the teeth in alveolar sockets, skeletal bones protect internal organs and acts as depot of mineral substances especially calcium (also is necessary for normal muscle function) [15]. Osteoporotic (OP) changes of skeletal bones occur because of impaired mineralization due to long term decreased uptake of vitamin D and calcium, increasing risk of fractures, accelerated jawbone resorption causes adentia [16, 17].

2. Vitamin D metabolism

There are 4 forms of vitamin D (calciferol) – lamisterol (vit. D_1), ergocalciferol (vit. D_2), cholecalciferol (vit. D_3) and dihydrotachysterol (vit. D_4), 2 of which being the most important – D_2 and D_3. Under the influence of UV radiation, ergosterol (plant based) and cholesterol (synthetized from 7-dehydrocholesterol of animal origin) are transformed into vitamin D_2 and D_3 respectively [1, 18]. Major part of vitamin D, around 90%, is synthesized in epidermis under the effect of the sun, while the rest is absorbed in small intestine together with food, nowadays being enriched with vitamin D with increasing frequency [19, 20]. Hydroxylation of vitamin D is a two-stage process, taking place in liver and kidneys. Thus renal and liver diseases impair metabolism of vitamin D. Enzyme 25-hydroxylase transforms vitamin D_3 into 25-hydroxyvitamin D_3 25(OH)D_3 in liver, which is the main metabolite of vitamin D_3, circulating in blood [21]. Recently it was proved that when inflammation occurs gingival fibroblasts and periodontal cells are capable of producing 25-hydroxylase and it appears to be a new extrahepatic site of 25(OH)D_3 synthesis [21]. Further, 25-hydroxyvitamin D_3 is hydroxylated in kidneys by means of 1 α-hydroxylase into 1,25-dihydroxyvitamin D (1,25$(OH)_2D_3$), calcitriol, active form of vitamin D, which is active hormone, participating in calcium absorption in intestines, important for both specific and nonspecific immune response against bacterial infections of oral cavity and other organs [2, 7, 21, 22]. Calcitriol binds to vitamin D-binding protein (DBP) and is transported to the cells of target tissues [23]. Synthesis of 1α-hydroxylase starts after receptors of cellular membranes have been influenced by microorganisms. Calcitriol is activated after binding to vitamin D receptors (VDR) of nuclei of immune and epithelial cells [7, 23–25]. VDR can be found in many human tissues, regulating activity of more than 200 genes in direct or indirect way [26, 27]. Numerous distribution of VDR in tissues determines complex effect of vitamin D, whereas deficiency determines disorders [26–28]. Polymorphism of VDR gene is related to many infectious diseases, including PD

[28–30]. Polymorphism of VDR gene and exact mechanism of periodontitis remain unclear so far. There is no link established between polymorphism of VDR genes and aggressive form of periodontitis [31, 32].

3. The role of vitamin D in immune response of periodontal tissues

Marginal gingival epithelium (GE) descends from free inner margin towards root apex and transits to sulcular epithelium – semi permeable membrane. Sulcular epithelium attaches to tooth by means of loose connections, creating favorable conditions for bacterial invasion from dental plaque [33]. Thin and non-keratinized epithelium forms a junctional epithelium (JE), laying at the bottom of gingival sulcus. JE is a narrow structure of 1–2 mm with good regenerative properties, connected to tooth by layer of organic substance. Its cubic and flat cells have gaps and attach to tooth and with each other by means of hemidesmosoms and have 2 basal membranes: internal, near the tooth, and the outer one on the other side, contacting with subepithelial tissue. Disruption of the bonds between enamel cuticle and JE leads to inflammatory processes. This anatomical unit plays a barrier function, which is supported by gingival fluid, flushing gingival sulcus – blood filtrate, containing lots of various protective cells (neutrophilic leucocytes, lymphocytes, monocytes, and macrophages), specific antibodies, immunoglobulins, cytokines, proteins, enzymes, epithelial cells and bacteria. The amount of gingival fluid can change due to circadian rhythms and depends on the health of PTs, oral hygiene, mechanic impact while mastication, medications used, etc. [34, 35]. Plasma proteins strengthen junctional epithelium-enamel bonds and namely calcitriol, affecting nonspecific immunity, activates synthesis of proteins, necessary for small adherens, gap and desmosome epithelium intercellular junctions, activates hydrogen peroxide secretion in monocytes, stimulates the synthesis of antimicrobial peptides, chemotaxis, production of cytokines and chemokines, cellular reproduction, vascular permeability, wound healing, and neutralization of bacterial endotoxins [7, 25, 36]. Hence $1,25(OH)2D_3$ enhances antibacterial defense of GE [25, 36, 37].

Immune response can be nonspecific and specific. After bacteria have entered the periodontal tissues, defensive mechanism starts – immune response, during which the foreign substance is neutralized and eliminated or memorized. Neutrophilic leucocytes play important role in nonspecific immune response. It is interesting, that these cells are always found in gingival sulcus and this is the only site in organism, where neutrophilic leucocytes can freely migrate from organism outwards. During the bacterial invasion into PTs, chemotactic mechanisms are activated and neutrophilic leucocytes start migrating from blood vessels into inflamed tissues. Right here, together with macrophages, they take part in phagocytosis, thus fighting with different antigens. Neutrophilic leucocytes, monocytes and activated macrophages produce immune mediators (IL-1β, IL-1ra, IL-6, IL-10, IL-12, TNF-α, PGE2, MMP, Interferon γ (IFNγ)) and chemokines [37]. Vitamin D protects the organism from excessive immune response by decreasing the secretion of IL-1, IL-6, IL-8, IL-12, TNF-α cytokines, decreases production of matrix metalloproteinase (MMP) in leucocytes [33, 38]. MMP – enzymes, participating in alterations of intercellular tissues. Blood plasma levels of MMP-3, MMP-8 and MMP-9 increase with PD [39]. Very high level of MMP-9 is detected in cases of rapidly progressing periodontitis, but it can decrease up to 69% taking even little doses of vitamin D for extended period of time [40, 41].

Blood monocytes, after migration into connective tissues, transform into macrophages, which are very important for both, cellular and humoral immunity, as they have surface receptors, reacting with any foreign substance [42]. In the process

of phagocytosis, immunoglobulins and serum complement envelope foreign substance. Such formation enters phagocytic vacuoles and is destroyed by lysosome enzymes. Afterwards these substances are transferred to lymphocytes, determining further immune response [42]. Products of tissue breakdown, histamine and complement system enhances phagocytosis. Specific immune response manifests later. Antibodies circulate in blood, T and B lymphocytes react only to specific antigen. Lymphocytes are often related to Langerhans cells of oral epithelium, producing cytokines, e.g., IL-1, activating T lymphocytes, enhancing proliferation and production of antibodies. Active T lymphocytes have cytotoxic effect. Keratinocytes of oral epithelium produce IL-1 and IL-8, regulating the amount of lymphocytes and polymorphonuclear leucocytes [42]. Impaired specific immunity is responsible for resorption, osteoclast genesis and inflammatory processes of bone, thus causing autoimmune diseases. Calcitriol suppresses progression of OP and PD with signs of autoimmune diseases.

Active hormonal form of vitamin D, $1,25(OH)_2D_3$, directly regulates antimicrobial immune response, modulates cytokine production and stimulates secretion of antimicrobial peptides by monocytes-macrophages and activates release of hydrogen peroxide in monocytes, thus exhibiting anti-inflammatory and antimicrobial properties [7, 43, 44]. Monocytes not only produce cytokines, but TNF-α as well, they release substances, activating lymphocytes and interacting with them as antigen-presenting cell (APC), destroy PTs, accelerate proliferation, differentiation and activation of osteoclasts, move in tissues in chemotactic way and participate in phagocytosis. Antimicrobial peptides play important role in nonspecific immunity against periodontitis causing agents. Antimicrobial peptides, β-defensines and especially cathelicidine LL-37 take part in neutralization of bacterial endotoxins, healing of wounds, regulate cell multiplication, blood vessel permeability, cytokine and chemokine production and chemotaxis, have prolonged antimicrobial activity and neutralize lipopolysaccharides as well [3, 7, 23, 25, 45, 46]. Activity of defensines depends on vitamin D levels [45, 46]. β-defensines show antimicrobial activity on PD bacteria– *Actinobacilus actinomycetemcomitans, Porphyromonas gingivalis, Fusobacterium nucleatum, Candida* and papilloma viruses [47]. APCs, including macrophages and dendritic cells, transform the main $25(OH)D_3$ form circulating in blood into active $1,25(OH)_2D_3$, and, via VDR, induce cellular response and regulate transcription. Antimicrobial activity via VDR is associated with cathelicidine hCAP-18 gene [48]. Cathelicidine has wide antimicrobial activity against gram-positive and gram-negative bacteria, some fungi and viruses [3]. Treatment with vitamin D increases cathelicidine mRNA level in keratinocytes, neutrophils and macrophages [3, 44]. Irritation of macrophage receptors with pathogens increases synthesis of $1,25(OH)_2D_3$, activity in macrophages and production of antibacterial proteins, cathelicidine and β-defensines, increases [43]. Therefore, it is supposed that vitamin D deficiency determines weak antibacterial response and tendency to infections [49].

During specific immune response, $1,25(OH)_2D_3$ affects B and T lymphocytes [7]. The latter release cytokines (interleukine-1, TNF-α, macrophage activating factor, macrophage migration inhibitory factor), stimulating resorption of dental supportive tissues (due to increased number of osteoclasts) and decay of extracellular matrix; release immunoglobulins; destroy pathogens, transferred by macrophages and dendritic cells and participate in production of antibodies. Such immune response mechanism enhances pathogenesis of PTs and aggravates the course of disease [16, 21, 33]. $1,25(OH)_2D_3$ suppresses proliferation, maturation and differentiation of dendritic cells [50]. T lymphocytes are one of the dominating cells in the beginning of PTs pathologic process and its regulator; immature

dendritic cells stimulate their tolerance, whereas mature dendritic cells activate them [51]. $1,25(OH)_2D_3$ decreases the number of APCs and ability of T lymphocyte to stimulate monocytes-macrophages [52]. The main target is T helpers. Vitamin D can contribute to formation of acquired immune response by selective stimulation of the specific T helpers [53]. B lymphocytes interact with macrophages and become plasmocytes, releasing Ig, which adhere to antigens. Such antigen–antibody complex activates complement system, initiating production of cytotoxic molecules, increasing blood vessel permeability, acting as chemotactic agents for polymorphonuclear leucocytes and macrophages. Vitamin D inhibits T lymphocyte proliferation, release of immunoglobulins and transformation of B lymphocytes into plasmocytes, hereby suppressing specific immune response, release of IL-1, IL-6, IL-8, IL-12 cytokines and alpha TNF-α [16, 21, 52]. IL-1, IL-6 and TNF-α are potential activators of osteoclasts and supporters of inflammation. IL-1 is released not only by afore mentioned monocyte-macrophages, but by endothelial and epithelial cells, fibroblasts and lymphocytes as well. This substance supports inflamation, stimulates production, differentiation and activation of osteoclasts, leading to resorption of alveolar bone, release of enzymes splitting extracellular matrix and release of E2 prostaglandin, enhancing relaxation of blood vessels and edema in PTs [13]. IL-6 activates synthesis of C reactive protein and glucose metabolism [54]. It is proven, that increase of vitamin D serum levels leads to decrease of IL-6 and leptin (factor indicating inflammation) levels, increase of adiponectin (cytokine inhibitor) and IL-8 levels [54, 55]. IL-8 activates neutrophil chemotaxis and is found in normal PTs. Its levels increase with the progression of inflammatory processes, therefore polymorphonuclear neutrophils are the first to react to inflammation. Its deficiency is related to severe forms of periodontitis. As the amount of pathogens increases, IL-8 levels and number of neutrophils increase as well, causing destruction of PTs [6]. Vitamin D acts on periopathogens, inhibits inflammation of PT and decreases IL-8 expression in periodontal ligament [55]. It can be concluded, that vitamin D plays important role in defensive mechanisms of PT and vital for the health of both soft and hard PTs.

4. Change in the concentration of 25-hydroxyvitamin D in plasma by periodontal diseases

There is no unanimous attitude towards the relation between these two factors as there exist differences between populations, test methodology and occurring limitations of the tests. When analyzing the relation between vitamin D, as protective factor, and PD, different criteria of vitamin D and examination of PTs are applied: average plasma level of vitamin D, dosage applied or any other certain diagnostic criteria. Status of PTs is evaluated according to the periodontal pocket depth, clinical attachment level, clinical attachment loss, attachment gain, alveolar bone loss, bone defects in oral cavity or any other selected criteria, such as short/long term results of periodontal surgery [56]. If only one side of oral cavity is examined, data cannot be accurate and the amount of information is lost. Studies can cover general population or part of it, e.g., smoking individuals, individuals of different age, with different PD, etc. Scientific base of linking these two factors and widening of the knowledge is possible due to widely chosen evaluative protocols and indicators. For example, smoking is risk factor for PD, but with the additional deficiency of vitamin D, destruction of PTs is more severe and more cases are identified [57]. Individuals over 50 year with low vitamin D levels have greater periodontal attachment loss than ones with high levels [58]. PTs in most cases are

healthier and risk is lower in individuals with sufficient levels of vitamin D, but it is not successfully proved in all the cases, leading to the controversial interpretation of the results obtained [56, 58–61]. It is becoming clear, that performing blood tests and monitoring vitamin D levels it is possible to suspect that individual is suffering from chronic periodontitis and condition of PTs is poor, especially in older population [56, 62, 63]. It can be one of additional diagnostic possibilities.

In case of acute inflammation, serum levels of 25-hydroxyvitamin D are increasing, as periodontal cells are producing it in the site of inflammation as anti-inflammatory agent. Serum levels of 25-hydroxyvitamin D usually are lower in cases of chronic inflammation. Such correlation might be explained that because of low serum levels of vitamin D, ability of epithelium to fight against pathogens is impaired and inflammation develops. Optimal serum level of 25-hydroxyvitamin D for prophylactic and therapeutic purposes should be 90-100 nmol/l, but it is not definitely clear what should be the daily dose of vitamin D in order to achieve these levels [13]. Gingival bleeding are the sign of both, acute and chronic PD. Decreased level of 25-hydroxyvitamin D correlates with worsened health status of PTs; course of chronic gingivitis (with insufficient serum levels) and intensified gingival bleeding [36, 58]. Gingival bleeding during the probing is observed by 20% less in patients with high serum levels of 25(OH)D, those with sufficient (≥ 50 nmol/L) levels of vitamin are less likely to develop PD by 33% and by 42% are less likely to have more than 50% of gingival bleeding [60, 64]. Prolonged combined supplementation of vitamin D and calcium decreases bleeding on probing, changes the clinical attachment level and pockets depth [65]. The tendency to severe periodontitis may decrease up to 33% with daily dose of 800 IU of vitamin D [62]. Exacerbation of gingival bleeding is observed during pregnancy, thus there are data, concerning the influence of vitamin D during this period (if 25(OH) D level < 75 nmol/L) [36]. Without timely evaluation of all these data, periodontitis of more severe form develops, as correlation between increased serum levels of vitamin D_3 and severity of PD exists, though it is not always confirmed [36, 66, 67]. There are cases described when serum level of 1,25(OH)D increases significantly following the periodontal treatment [68]. Besides, increased serum level of 25(OH)D is common in patients with aggressive periodontitis (AP)- disease of PTs, affecting young individuals, characterized by rapid destruction of PT and tooth loss. Significantly higher vitamin D binding protein (DBP), IL-6, procalctonine and 25(OH)D_3 plasma levels and higher counts of leucocytes and neutrophils are found in patients with this PD [69]. Level of 25(OH)D_3 increases due to activation of 25-hydroxylase in periodontal cells in acute inflammation of PTs, and decrease in chronic one [67]. Due to production of this enzyme in cases of AP, levels of 25(OH)D_3 in gingival sulcus is up to 300 times higher than in blood plasma [21]. DBP is plasma protein and is synthesized by hepatocytes [70]. It is the main carrier of 25(OH)D_3 in the plasma, directly affecting cellular functions, including activity of macrophages [71, 72]. DBP bound to the cell surface, B-lymphocytes, T-lymphocytes, monocytes, neutrophils [70]. The amount of this protein is related to the severity degree of illness with direct relation to neutrophil – increased number of neutrophils and IL-6 can contribute to increased plasma levels of DBP by active periodontitis. DBP is the most important "during inflammation since it induces selective recruitment of neutrophils" [70]. Activated neutrophils can excrete DBP, which expression is regulated by IL-6, participating in immune response [69]. Thus detection of plasma levels of DBP could confirm correlation between it and periodontal inflammation. Anti-inflammatory effect depends on the dose of vitamin supplement. Safe and effective anti-inflammatory dose of 500–2000 IU of vitamin D is recommended. Results are noted earlier when higher dose of 2000 IU is used [73].

5. Conclusion

In conclusion, vitamin D is very unique substance due to its abilities, functions and participation in various processes. Its optimal serum levels could prevent occurrence of numerous diseases, including such common diseases throughout the world as chronic periodontal diseases, which are social problems, compromising individual's quality of life.

Conflict of interest

The author declares no conflict of interest.

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