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REVIEW ARTICLE

Correlation of Salivary Cytokine Il-17a and 1,25 Dihydroxycholecalciferol in Patients Undergoing Orthodontic Treatment-A Review

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ABSTRACT
Orthodontic treatment is associated with changes in the oral environment, including alterations in salivary biomarkers. This review aims to explore the correlation between salivary cytokine IL-17A and 1,25 dihydroxycholecalciferol (1,25(OH)2D3) levels in patients undergoing orthodontic treatment. IL-17A, a proinflammatory cytokine, and 1,25(OH)2D3, the active form of vitamin D, play crucial roles in immune regulation and bone metabolism, respectively. Understanding their interplay during orthodontic treatment could provide insights into the inflammatory and bone remodeling processes involved. Through a comprehensive review of the literature, this paper examines the existing evidence on the relationship between IL-17A and 1,25(OH)2D3 levels in saliva during orthodontic interventions. Insights from this review may contribute to elucidating the mechanisms underlying orthodontic treatment outcomes and potentially guide the development of novel therapeutic strategies to optimize patient care.
Keywords: *IL-17A, Orthodontic tooth movement, Vitamin D*

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INTRODUCTION

Orthodontics is a dental specialty focused on monitoring and managing the development and growth of the teeth and related anatomical structures, from birth until the teeth are fully mature. This field encompasses both preventive and corrective procedures to address dental irregularities, which often require repositioning teeth using either functional or mechanical methods to achieve normal occlusion and improve facial aesthetics [1]. The goals of orthodontic treatment are well illustrated by Jackson's triad, which includes three primary objectives: functional efficiency, structural balance, and aesthetic harmony. Although there have been significant advancements in simulating treatments and the biomechanics involved in correcting malocclusions, the duration of treatment remains a major concern for both clinicians and patients. Biological, biomechanical, physical, and surgical approaches were explored to reduce the treatment time. The biological methods to accelerate Orthodontic Tooth Movement (OTM) involve the use of molecules such as Prostaglandin (PG), Interleukin (IL), Receptor Activator of Nuclear Factor Kappa B Ligand (RANK & RANKL), Osteoprotegerin (OPG), Vitamin D, Parathyroid Hormone (PTH), and Relaxin. Although experimental studies on animals using these molecules, alone or in combination, have shown promising results, human studies remain limited due to challenges with patient compliance [2][3]. This review aims to examine the available evidence on the roles of salivary cytokine IL-17 A and 1,25-dihydroxycholecalciferol in accelerating orthodontic tooth movement.

Saliva as a Diagnostic Tool in Orthodontic Tooth Movement

Early detection of diseases is critical for effective diagnosis and treatment planning in clinical practice. Both clinicians and researchers are continually searching for innovative diagnostic methods that allow for the early identification of diseases. Traditional diagnostic procedures often involve invasive techniques, such as blood draws or biopsies, which can cause discomfort and stress for patients. Various biomarkers are utilized to facilitate early detection and prevention of human diseases. Saliva offers a compelling alternative to serum due to its non-invasive nature, safety, and ease of collection while containing adequate amounts of biomarkers necessary for disease diagnosis. Compared to gingival crevicular fluid (GCF) collection, saliva sampling is less sensitive to technique [4]. Although the use of saliva for diagnostic purposes dates back to the 1960s, significant advancements have been made in sensitive detection techniques since then. Salivary diagnostics now play a crucial role in clinical disease diagnosis. Currently, saliva is employed in diagnosing oral, autoimmune, and genetic disorders, detecting infections and malignancies, monitoring hormone and drug levels, serving as forensic evidence, and, notably in orthodontics, as an indicator of bone turnover [5]. According to the National Institutes of Health Biomarkers Definitions Working Group, the definition of a biomarker is "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathological processes, or pharmacologic responses to a therapeutic intervention" [6][7]. An ideal biomarker should possess specific characteristics: it must be valid, safe, easily measurable, and collected through non-invasive methods. Additionally, it should have high sensitivity to accurately identify individuals with a disease (true-positive) and those without (true-negative) [6]. Consistency across various demographics, including age, gender, and ethnicity, is also essential. The human oral cavity contains several reliable biomarkers, including saliva, gingival crevicular fluid, peri-implant sulcular fluid (PISF), and mouth rinse remnants. Saliva, rich in diverse biomarkers, can be easily collected without causing trauma and stored in larger quantities. This review will discuss the relevance of saliva as a biomarker and its role in orthodontic tooth movement.

Formation and Function of Saliva

Saliva, once primarily viewed as a digestive fluid, is now recognized as a complex biofluid containing a wide array of molecular, microbial, and chemical components. These components can reflect underlying infectious, local, or systemic conditions[9-11], thus highlighting the role of saliva in molecular diagnostics. In the human oral cavity, saliva is sourced mainly from three major salivary glands—the parotid, submandibular, and sublingual glands—alongside numerous minor salivary glands. Acinar cells in these glands synthesize saliva, which is then transported through small ducts into the oral cavity[12]. The major salivary glands are responsible for 90% of saliva production, with the remaining 10% coming from the minor glands, such as labial, buccal, lingual, and palatal glands. Saliva is a slightly acidic fluid, with a pH ranging from 6 to 7. It predominantly consists of water (99%), followed by proteins (0.3%) and inorganic substances (0.2%)[13]. Humans typically produce about 1 to 1.5 liters of saliva daily, with secretion rates varying from 0.3 to 0.7 ml per minute [14,15]. The functions of saliva extend beyond digestion to include tasting, swallowing, tissue lubrication, and acting as a protective barrier against pathogens. Saliva contains various electrolytes such as sodium, potassium, magnesium, calcium, bicarbonate, and phosphates. It also harbors proteins, immunoglobulins, enzymes, mucins, and nitrogenous compounds like urea and ammonia. The interaction of these components supports several key functions: bicarbonates and phosphates help modulate saliva's pH and buffering capacity; mucins and protein macromolecules aid in cleansing and aggregating oral microorganisms; calcium and phosphate play roles in demineralization and remineralization; and immunoglobulins, enzymes, and proteins contribute to antibacterial actions.[14] The high permeability of salivary glands, due to their capillary coverage, allows for the exchange of molecules between blood and saliva.[16] These molecules can be transported through transcellular (active and passive) or paracellular (extracellular) routes, potentially altering the molecular composition of saliva.[17,18] This property suggests that saliva may carry vital information about an individual's health status.

Efficacy of Saliva over Blood and GCF

Blood is a well-known source for measurable biomarkers, containing a wide range of hormones, antibodies, enzymes, and growth factors.[19] However, saliva presents several advantages over blood:

1. It is easily and safely collected, even allowing for self-collection.
2. Collection is non-invasive, reducing patient discomfort and compliance issues.
3. Saliva is safer to handle due to the absence of bloodborne pathogens [20]
4. Saliva is easier to store and ship because it does not clot.
5. It is more economical since collection, transport, and storage are less expensive.

Despite these advantages, saliva also has some drawbacks. Variations in its volume and composition can occur due to physiological and pathological states, both between individuals and within the same person over time, which complicates standardization.[21] Additionally, the concentration of analytes in saliva tends to be lower compared to blood. For example, salivary IgA levels range between 250 to 500 μg/ml, whereas serum levels are 2.5 to 5 mg/ml[22]

Gingival crevicular fluid (GCF) is another oral fluid, found in the crevice between the tooth surface and epithelial tissue. The nature of GCF—whether it's a transudate or exudate—remains controversial, and its flow rate varies with the degree of inflammation, typically ranging from 0.5 to 2.4 mL. [23] GCF also presents challenges, including variability in fluid amount between healthy and diseased states, influencing collection time, potential contamination, and difficulties in extracting molecules from collected samples.

Methods of Collection of Saliva

Salivary sample collection is influenced by several factors, including gland type, salivary stimulus (gustatory or mechanical), circadian rhythm, diet, age, physiological state, and the method of collection. Common collection methods include draining, suctioning, and spitting, with rinsing the oral cavity beforehand to avoid contamination being crucial. The composition of submandibular saliva is notably lower in the afternoon due to circadian variations, similar to findings in studies on the parotid gland . For accurate results, it is advised to avoid eating, drinking, or brushing teeth at least two hours prior to collection.[24,25] Stimulated saliva can be obtained by chewing unflavored gum or wax for five minutes, with rates expressed in ml/min. Stimulated salivary flow rates below 0.7 ml/min are indicative of xerostomia. Studies show that different methods of saliva collection and variations in oral hygiene practices can significantly affect salivary biomarker levels, emphasizing the need for standardized methods to optimize saliva's use in diagnostics.[26] .

Salivary Diagnostics

Salivary molecules provide crucial diagnostic information and can be examined through proteomic, genomic, and transcriptome analyses.

Proteomic Analysis

Human saliva contains proteins synthesized within the salivary glands and those derived from blood. Around 2,340 salivary proteins have been identified, with 20-30% overlapping with blood proteins. Recently, a comprehensive list of 1,166 salivary secretory proteins was published [27,28]

Genomic Analysis

Saliva has emerged as an alternative to blood for genomic RNA collection, meeting all requirements for sample collection in genetic studies.[29]

Transcriptome Analysis

Salivary transcriptome analysis is valuable for diagnosing diseases. A typical salivary transcriptome contains about 3,000 mRNA molecules, with 180 common across healthy individuals, forming the normal salivary transcriptome core (NSTC).[30, 31] .

Salivary Biomarkers and Their Role in Orthodontic Tooth Movement (OTM)

Salivary proteins show promise for monitoring the effectiveness and adverse reactions to orthodontic treatment.[32] The ability to see the same set of patients over time allows for easy collection and measurement of saliva at various points, minimizing individual variation. [33]. Myeloperoxidase (MPO), an enzyme found in polymorphonuclear neutrophil granules, serves as a marker for inflammation associated with orthodontic tooth movement, with activity peaking two hours after initial activation and remaining elevated for up to seven days.[34] Leptin, which acts both as a hormone and a cytokine, plays a role in bone remodeling, resorption, and new bone formation. Studies have established a positive correlation between salivary leptin levels and the rate of tooth movement, with reduced rates observed in overweight individuals compared to those with normal weight.[35] The evaluation of osteotropic factors, such as sRANKL and OPG, in saliva revealed that their ratios might correlate with different OTM phases, although their protein concentrations were not directly associated. ApoE, a protein involved in fat metabolism, was found to be upregulated in active orthodontic cases, suggesting lower bone metabolism and increased adipogenesism [36,37] Challenges in proteomics include low protein concentrations, transient secretion of biomarkers, and potential contamination by food, oral mucosa, or microorganisms . Certain biomarkers identified may relate more to bone healing post-surgery rather than to orthodontic treatment directly[38] Salivary levels of deoxypyridinoline (DPD) and bone-specific alkaline phosphatase (BAP) are indicators of bone remodeling, with DPD prominent during early OTM phases and BAP indicating bone formation cessation . Increases in sRANKL and decreases in OPG levels after orthodontic activation support their potential role in monitoring OTM [39] Assessment of cytokines and enzymes in

saliva and blood via multiplex enzyme-linked immunosorbent assay (ELISA) has shown that saliva is more sensitive than blood in detecting changes secondary to orthodontic treatment . Salivary parameters like pH, antioxidants, C-reactive protein (CRP), cortisol, and protein levels largely remain stable post-exposure to orthodontic appliances, with minor transient effects.[40-45] Increased alpha-amylase activity following orthodontic activation indicates stress response in patients, linked to pain and masticatory performance . Biochemical properties such as protein concentration and calcium levels show significant changes during OTM, affecting the oral environment[45-48]

Orthodontic Tooth Movement

Orthodontic Tooth Movement (OTM) involves a complex biological process that occurs when orthodontic forces are applied to teeth. This process creates areas of pressure and tension in the periodontal ligament (PDL), leading to both macroscopic and microscopic changes dependent on the force's magnitude, frequency, and duration. Applied forces induce strain in the PDL, enhancing its blood supply and triggering the release of various biochemical mediators like cytokines, growth factors, neurotransmitters, and other substances. These mediators facilitate bone remodeling, either by promoting bone deposition or resorption, thereby enabling tooth movement.

Historical Perspective of Orthodontic Tooth Movement

The study of orthodontic tooth movement began in earnest in 1911. Oppenheim was among the first to examine histological changes during tooth movement in baboons. Reitan later expanded on this research, developing the pressure-tension model, which suggests that orthodontic forces affect each side of the tooth differently. Another significant theory, the hydraulic theory, proposed by Stuteville in 1938, posits that tooth movement forces are absorbed by fluids from various body systems, which helps regulate and limit movement. Subsequent work by Bien and Baumrind in the 1960s further explored these theories. While both the pressure-tension and fluid flow concepts have their merits, further research is needed to understand the mechanisms of tooth movement fully.

Theories of Tooth Movement

Two main theories explain orthodontic tooth movement:

The Pressure-Tension Theory

This theory posits that tooth movement occurs in the PDL space, creating pressure and tension sides. On the pressure side, reduced blood flow leads to decreased cell replication and disorganization of the PDL, while the tension side experiences increased cell activity and fiber production due to stretching. Introduced by Sandstedt in 1904 and refined by Oppenheim and Schwarz in 1932, this theory highlights the differential responses in the PDL. Excessive force can cause bone resorption and tissue damage. Histological studies show that the initial signs of tissue damage include cell death and formation of cell-free zones. Macrophages and osteoclasts then remove the damaged tissue, leading to bone resorption and remodeling. The pressure-tension theory indicates that osteoclasts are involved in resorbing bone on the pressure side, while osteoblasts promote new bone formation on the tension side.[49-55]

The Bone Bending Theory

Proposed by Farrar in 1988 and later refined by Baumrind and Grimm, this theory suggests that orthodontic forces cause deflection in the alveolar bone, resulting in strains in the PDL and stimulating bone turnover. According to this theory, bone responds to orthodontic forces by bending and undergoing reorganization[56-60]. The concept also includes the generation of electric potentials in stressed tissues, which influence bone remodeling. Research by Bassett and Becker in 1962 supported the idea that bioelectric responses play a role in orthodontic tooth movement. Despite these findings, further research is needed to clarify the exact mechanisms.[61-64]

Phases of Tooth Movement

Tooth movement is divided into three phases:

Initial Phase: Characterized by rapid tooth displacement within the PDL following force application. This phase lasts 1-4 days and involves the initiation of osteoclastic and osteoblastic activities.

Lag Phase: This phase follows the initial phase and is marked by minimal movement due to the hyalinization of the PDL in compression areas. Lasting from 4 to 20 days, it involves the removal of necrotic tissue before further tooth movement can occur.

Post-Lag Phase: Starting approximately 40 days after force application, this phase involves gradual or rapid tooth movement and ongoing bone remodeling. Collagen fibers show irregular orientation and bone resorption occurs on the pressure side, while new bone formation by osteoblasts is evident on the tension side.[65-70]

Factors Affecting Tooth Movement

Several factors influence the rate and efficiency of orthodontic tooth movement:

Inflammatory Response: The application of orthodontic forces triggers an acute inflammatory response, increasing PDL blood flow and leukocyte exudation. This inflammatory process leads to the production of prostaglandins, growth factors, and cytokines, which are crucial for bone remodeling.

Arachidonic Acid Metabolites: Prostaglandins (PGs) and leukotrienes (LTs), derived from arachidonic acid, are essential in mediating tooth movement. PGE2 enhances osteoclast activity and bone resorption, while leukotrienes may influence tooth movement [71].

Prostaglandins: Originally discovered in human semen and identified in various cell types, prostaglandins like PGE2 are key mediators in orthodontic tooth movement. They stimulate osteoclast differentiation and activity, facilitating bone resorption and promoting osteoblastic differentiation and bone formation.[72-79]

Cytokines and Their Role in Orthodontic Tooth Movement

Cytokines are a diverse group of signaling molecules, including proteins, peptides, and glycoproteins, secreted by specific immune cells. They act on adjacent cells at low concentrations through autocrine or paracrine mechanisms, facilitating cell-to-cell communication.

Cytokines in Bone Remodeling

Various cytokines, including interleukins (IL) such as IL-1, IL-2, IL-3, IL-6, IL-8, tumor necrosis factor-alpha (TNFα), gamma interferon (IFNγ), and osteoclast differentiation factor (ODF), are central to bone metabolism and orthodontic tooth movement (OTM). IL-1, for example, activates osteoclasts via the IL-1 type 1 receptor, with its release triggered by stimuli like neurotransmitters, bacterial products, cytokines, and mechanical forces [81, 82]. Specifically, IL-1 directs osteoclasts to resorb bone by targeting osteoblasts, and increased IL-8 levels at periodontal ligament (PDL) tension sites are believed to support this remodeling process [83]. Furthermore, IL-1 activates NF-kappaB-like factors in osteoclast-like cells [84]. TNFα promotes the differentiation of osteoclast progenitors into mature osteoclasts in the presence of macrophage colony-stimulating factor (M-CSF) [85][86]. Studies using histochemical staining have shown elevated IL-1 and TNFα levels in the PDL and alveolar bone during OTM in animal models [86][87]. IFNγ, a critical cytokine in innate and adaptive immunity, activates macrophages and modulates the production of IL-1 and TNFα. During OTM, IFNγ contributes to bone resorption by inducing the apoptosis of effector T-cells [88]. Research has consistently shown that pro-inflammatory cytokines like IL-1β, IL-6, and TNF-α are pivotal in bone remodeling, driving both bone resorption and formation. In animal studies, mRNA expression of IL-1β, IL-6, and TNF-α peaked three days after the application of orthodontic forces, reflecting their role in initiating bone resorption [89]. The RANK/RANKL/OPG signaling pathway, crucial for osteoclast differentiation and activity, regulates bone remodeling and repair. RANK, a receptor for RANKL (RANK-Ligand), plays an essential role in this process. Osteoprotegerin (OPG), also known as osteoclastogenesis inhibitory factor (OCIF), is a cytokine receptor that acts as a decoy receptor for RANKL, inhibiting osteoclastogenesis and bone resorption [90]. OPG expression can be upregulated by IL-1β, TNFα, 25(OH)2D3, and estrogen [91-93]. RANKL, produced primarily by osteoblasts, is essential for activating osteoclasts and promoting bone resorption. Recent research has identified osteocytes as a major source of RANKL during bone remodeling [94-96], with RANKL from other cell types contributing to bone loss in inflammatory conditions. A study by Garlet TP et al. utilized RT-PCR to examine the expression of various cytokines and bone remodeling markers in the PDL during rapid maxillary expansion in humans. The study found a differential expression of cytokines between the compression and tension sides of the PDL. On the compression side, TNF-α, RANKL, and matrix metalloproteinase-1 (MMP-1) were elevated, while on the tension side, IL-10, tissue inhibitor of metalloproteinases-1 (TIMP-1), OPG, and osteocalcin (OCN) levels were higher. TGF-β levels were similar on both sides.

Role of IL-17A in Tooth Movement

Interleukin-17A (IL-17A), commonly referred to as IL-17, was first identified by Rouvier et al. in 1993 from a rodent T-cell hybridoma [98]. IL-17 is part of a cytokine family comprising six ligands: IL-17A, IL-17B, IL-17C, IL-17D, IL-17E (IL-25), and IL-17F. These ligands interact with six receptors, including IL-17RA, IL-17RB/IL-17R, IL-17RC, IL-17RD/SEF, and IL-17RE [101]. While IL-17 was initially thought to be secreted exclusively by T cells [101, 102], it is now understood to be produced by several immune cells, including macrophages, natural killer cells, dendritic cells, and other lymphoid cells. CD4+ T cells, CD8+ T cells, and innate lymphoid cells (ILCs) are major producers of IL-17A [103]. The differentiation of IL-17-producing CD4+ T cells, known as Th17 cells, is driven by cytokines such as IL-1, IL-6, and transforming growth factor-beta (TGF-β) [108]. In addition to IL-17A, Th17 cells also produce cytokines like IL-17F, TNF-α, IL-21, and IL-22, all of which contribute to bone resorption and collagen degradation in vitro [109]. IL-17A stimulates the expression of RANKL (Receptor Activator of Nuclear Factor Kappa-B Ligand) in osteoblasts [110]. Th17 cells, which exhibit high RANKL levels, play a significant role in enhancing bone

turnover [111]. IL-17A, encoded by the IL17A gene, is a pro-inflammatory cytokine that regulates key signaling pathways such as NF-kappa B and mitogen-activated protein kinases. It also induces the production of IL-6, making it crucial for paradental tissue remodeling during orthodontic tooth movement.

Methods for Detecting Salivary Cytokines

Bioassays: These assays have historically been used to measure cytokine bioactivity in specific biological models or cell lines. Examples of bioassays include tests for chemotactic activity, proliferation, and cytotoxicity. Although bioassays offer good sensitivity and the ability to detect biologically active molecules, they have limitations such as lack of specificity, extended processing times, and imprecision.

Immunoassays: Immunoassays, particularly enzyme-linked immunosorbent assays (ELISA), are currently the preferred method for cytokine detection. ELISA uses a primary antibody for cytokine capture and a secondary antibody that is either enzyme- or radioisotope-conjugated for detection. Traditional ELISA kits are designed to detect one cytokine at a time. However, multiplex systems now allow for the simultaneous measurement of multiple cytokines in a single assay. These systems can analyze cytokine levels in various body fluids, including plasma, serum, cell lysates, cerebrospinal fluid, ascites, saliva, and urine.

Flow Cytometry: Flow cytometry, along with techniques like high-performance liquid electrophoresis, is another method employed for detecting cytokines and their receptors. Flow cytometry is particularly effective in analyzing intracellular cytokines and can provide results in under two hours. Despite its efficiency, this method presents challenges such as difficulties in quantifying intracellular cytokines, dealing with background autofluorescence, and ensuring proper gating and negative controls [112].

Role of 1,25-Dihydroxycholecalciferol in Orthodontic Tooth Movement

1,25-Dihydroxycholecalciferol (1,25-DHCC), or calcitriol, is the active form of vitamin D, primarily synthesized in the kidneys [113, 114]. It plays a critical role in calcium homeostasis and functions as a hormone that binds to the vitamin D receptor (VDR) in the nucleus of cells, enhancing gene expression. Calcitriol raises blood calcium levels by promoting its absorption in the intestines. In response to low serum calcium, it stimulates parathyroid hormone (PTH) secretion, which in turn increases phosphate ion excretion and boosts calcium reabsorption in the kidneys. PTH also facilitates the conversion of 25-hydroxycholecalciferol into 1,25-dihydroxycholecalciferol. Additionally, 1,25-DHCC stimulates osteoclast differentiation and activation, critical for bone resorption, and also supports bone mineralization and osteoblastic differentiation in a dose-dependent manner [78].

Historical Perspective of 1,25-Dihydroxycholecalciferol

Vitamin D is among the first hormones discovered, initially identified in phytoplankton species such as ***Emiliani huxleyi*** [115], which synthesizes vitamin D when exposed to sunlight. It is vital for calcium maintenance across marine and terrestrial life. Vitamin D production is influenced by factors like skin pigmentation, seasons, latitude, altitude, and sunscreen use. A deficiency in vitamin D has been linked to systemic diseases, including cardiovascular disorders, diabetes, and immune deficiencies. In orthodontics, vitamin D is known to aid in enhancing tooth movement and stabilizing the position of teeth. The human body requires approximately 3,000-5,000 IU of vitamin D daily [116], which can be derived from food sources like eggs and fatty fish [117, 118]. Non-vegetarians typically have higher vitamin D levels than vegetarians. An inverse relationship exists between vitamin D levels and serum PTH concentration, with a 25OHD level of about 20 ng/ml considered adequate for suppressing PTH [119].

Metabolism of 1,25-Dihydroxycholecalciferol

After ingestion, vitamin D is transformed into chylomicrons that enter the bloodstream via the lymphatic system [120]. It binds to vitamin D-binding proteins and lipoproteins for transport to the liver, where vitamin D2 and D3 undergo hydroxylation to produce a metabolite that reflects the body's vitamin D status. This metabolite is further hydroxylated in the kidneys, producing the active hormone calcitriol [121, 122]. The enzyme 1α-hydroxylase, mainly located in the proximal tubules of the kidney, converts 25-hydroxyvitamin D into 1,25-dihydroxyvitamin D. This metabolite has been detected in human gingival fibroblasts and periodontal ligament cells, indicating potential autocrine or paracrine functions in these tissues. Sunlight is the main source of vitamin D, converting it into previtamin D3, tachysterol, and lumisterol through photoconversion. UVB radiation facilitates the formation of vitamin D3, while lumisterol and tachysterol help prevent vitamin D overproduction during prolonged sun exposure. Vitamin D is essential for maintaining serum calcium and phosphate levels, critical for bone mineralization, muscle contraction, nerve function, and preventing hypocalcemic tetany. Vitamin D aids in absorbing approximately 40% of calcium and 80% of phosphorus with the help of 1,25(OH)2D [123]. Vitamin D3, produced in the skin, must undergo further metabolic steps to become active. It is first

hydroxylated in the liver, followed by 1α-hydroxylation by the enzyme CYP27B1, which forms 1,25(OH)2D, the most potent vitamin D metabolite [124].

Cutaneous Production of Vitamin D3

Studies by Holick et al. showed that pre-D3 production under UV irradiation occurs rapidly, reaching peak levels within hours. UV exposure also converts pre-D3 into lumisterol and tachysterol. Although pre-D3 levels peak early, lumisterol continues to accumulate with extended UV exposure, while tachysterol does not accumulate significantly [125].

Renal Production of 1,25(OH)2D

Vitamin D's hormonal effects are mainly mediated by 1,25(OH)2D, its most potent metabolite. The enzyme CYP27B1 converts 25OHD into 1,25(OH)2D, expressed not only in epidermal keratinocytes and renal tubules but also in other tissues such as the brain, placenta, testes, intestines, lungs, breast, and macrophages. The kidney remains the primary source of circulating 1,25(OH)2D, though extra-renal CYP27B1 production can meet local needs. CYP27B1 activity is regulated by factors such as PTH, FGF23, calcium, phosphate, and 1,25(OH)2D itself. Cytokines like IFN-γ and TNF-α can stimulate extra-renal production, though the exact mechanism behind PTH regulation remains unclear.

Mechanism of Action

1,25-DHCC elevates blood calcium levels by:

- Promoting dietary calcium absorption in the gastrointestinal tract.
- Enhancing renal calcium reabsorption, reducing urinary calcium loss.
- Stimulating calcium release from bones.

Calcitriol stimulates osteoblasts to release RANKL, which activates osteoclasts. In cooperation with PTH, calcitriol indirectly promotes osteoclast activity by increasing phosphate excretion from the kidneys, leading to bone demineralization and increased serum calcium levels. PTH also stimulates calcitriol production. Calcitriol exerts its effects through the VDR, which, upon binding to calcitriol, moves to the nucleus to enhance the expression of genes responsible for producing calcium-binding proteins, improving the absorption of calcium and phosphate from the intestines. Although calcitriol stimulates bone resorption, its role in increasing serum calcium results in overall bone accumulation due to the enhanced intestinal calcium absorption [126-129]. Additionally, calcitriol inhibits the release of calcitonin, a hormone that decreases blood calcium by suppressing bone resorption.

1,25-Dihydroxycholecalciferol and Orthodontic Tooth Movement

Research has shown that local administration of 1,25-DHCC and PGE2 in rats significantly increases the rate of orthodontic tooth movement (OTM) compared to controls. 1,25-DHCC is more effective than PGE2 in bone remodeling during OTM due to its balanced influence on bone formation and resorption. Local injections of 1,25-DHCC stimulated bone formation, aiding in the re-establishment of supporting structures, particularly the alveolar bone, after orthodontic treatment. Vitamin D promotes osteoclast differentiation and activity, leading to increased bone resorption and faster OTM [133, 134]. Early studies demonstrated that injecting vitamin D metabolites increased osteoclastic and osteoblastic activity throughout the experiment. Intraligamentary injections of vitamin D metabolites enhanced osteoclast activity, resulting in faster canine retraction. 1,25-DHCC administration increased the presence of osteoblasts on the external alveolar bone surface, accelerating OTM more effectively than PGE2 [135-138]. Beyond promoting new bone formation, calcitriol stabilizes teeth post-OTM. A temporary increase in bone resorption occurs within the first two days of calcitriol administration, followed by bone formation after 14 days. In orthodontic patients with vitamin D deficiency, OTM may be slower, with younger patients exhibiting faster tooth movement and higher osteoclast counts than older individuals. Enhanced canine distalization and reduced cancellous bone density have been observed with local 1,25-DHCC administration, leading to improved OTM rates. A strong correlation between serum and salivary vitamin D levels underscores its importance in orthodontic treatment. Additionally, vitamin D modulates immune responses in periodontal cells, reducing inflammatory responses while promoting anti-inflammatory cytokine secretion, supporting its role in accelerating OTM [139, 140].

Table 1: Table showing comparison of characteristics between blood and saliva sample collection.

Characteristics	Blood	Saliva
Storage	Refrigeration required for stability	Stable at higher temperatures
Patient compliance	Phlebotomy required	Self-administered
DNA quantity	4.5 to 11x105 WBS yielding 10-18µg/ml of genomic DNAHigh amount	4.3x105 cells/ml (Epithelial or leucocytes) DNA Good amounts
DNA quality	High with minimal contamination	Contamination commonly found
Genome wide analysis	High call rate	High call rate
DNA methylation analysis	Good	Comparable with blood
PCR analysis	Good analysis	Good analysis

Table 2. Table showing a comparison cAMP and cGMP pathways

	cAMP	cGMP
Stimuli	Hormonal & Mechanical	Intracellular regulator of endocrine & non endocrine
Action	Phosphorylation of specific substrate proteins by its dependent protein kinases	Specific substrate proteins by cGMP-dependent protein kinases
Alteration in level	Synthesis of polyamines, nucleic acids, proteins and secretion of cellular products	Synthesis of nucleic acids, proteins and secretion of cellular products.

CONCLUSION

In summary, the relationship between salivary cytokine IL-17A and 1,25-dihydroxycholecalciferol (1,25(OH)2D3) levels during orthodontic treatment reflects complex interactions between inflammatory responses and bone metabolism. Current literature suggests that orthodontic interventions influence salivary IL-17A and 1,25(OH)2D3 levels, which correlate with local immune and bone remodeling processes. However, inconsistencies and gaps in the evidence necessitate further research to clarify these mechanisms and their clinical implications. Future studies should focus on longitudinal investigations with larger sample sizes to better understand the dynamics of IL-17A and 1,25(OH)2D3 throughout orthodontic treatment stages. Additionally, exploring the link between salivary biomarker levels and treatment.

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