Chapter

Orthodontic Therapeutic Biomarkers in Saliva and Gingival Crevicular Fluid

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Abstract

Several biologically active substances representing the bone deposition and resorption processes are released following damage to periodontal tissue during orthodontic movement. Biomarkers are by definition objective, quantifiable characteristics of biological processes. The analysis of saliva/salivary fluid and Gingival crevicular fluid (GCF) may be an accepted way to examine the ongoing biochemical processes associated with bone turnover during orthodontic tooth movement and fixed orthodontic treatment pain. Assessing the presence of these salivary physiological biomarkers would benefit the clinician in appropriate pain diagnosis and management objectively of various problems encountered during the orthodontic procedures and for better outcome of biomechanical therapy. Due to lack of standardized collection procedure, even though well accepted by patients, saliva is often neglected as a body fluid of diagnostic and prognostic value. A literature search was carried out in major databases such as PubMed, Medline, Cochrane library, Web of Science, Google Scholar, Scopus and EMBASE for relevant studies. Publication in English between 2000 to 2021 which estimated Saliva markers as indicators of orthodontic tooth movement was included. The list of biomarkers available to date was compiled and is presented in table format. Each biomarker is discussed separately based on the available and collected evidences. Several sensitive salivary and GCF biomarkers are available to detect the biomechanical changes occurring during orthodontic tooth movement and pain occurring during fixed orthodontic therapy. Further focussed research might help to analyze the sensitivity and reliability of these biomarkers or cytokines, which in turn can lead to the development of chairside tests to assess the pain experienced by patients during orthodontic therapy and finally the outcome of the fixed orthodontic therapy.

Keywords: fixed orthodontic therapy, molecular biomarkers, saliva, GCF, objectivity

1. Introduction

Biomarkers are—quantifiable criteria of biological processes that provide indications objectively. During the orthodontic procedure, the analysis of saliva/salivary

fluid and Gingival crevicular fluid (GCF) may be examined to monitor biological process/progress.

In the orthodontic treatment, emphasis is on the point of patient care for the best results, by growth modification of the craniofacial region along with alveolar bone remodeling during fixed orthodontic procedure [1]. Induction of biologically active compounds occur inside the periodontium due to the orthodontic treatment which eventually induces cellular response in different microenvironment for biological response [1]. Orthodontic treatment is considered successful by three major factors namely periodontal health, oral hygiene and optimal orthodontic forces [1, 2]. Availability of newer techniques have reduced lateral effects like, pain, periodontal diseases, abbreviate the treatment period and limit iatrogenic damages like root resorption and development of nonvital teeth. The sequential events that occur following the orthodontic tooth movement (OTM) can be illustrated by the released molecules, regarded as biomarkers [1, 2]. Biomarkers are attributes that can be quantified, these can the indicators of biological processes which may be normal or pathogenic and/or other metabolic processes [3, 4]. Other important features of the biomarkers are specificity and sensitivity, which will have the ability to notify the biological conditions/changes occurring during any process/procedures [4]. Adequate knowledge about the cellular and biological processes makes it easier to understand the biological mechanics that can shorten the treatment time avoiding the detrimental effects linked to the orthodontic treatment due to its objective characteristics [3, 4].

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Forces induced by orthodontic therapy stimulates periodontium cells to release many chemical intermediaries like cytokines. The cytokines contribute immensely in the periodontal and alveolar bone remodeling, bone resorption and new bone deposition [5]. During the process of bone metabolism, the biomarkers are released into the circulation which indicates bone remodeling activity comprising of both osteoblastic deposition and osteoclastic resorption. Systemic circulation in the orthodontic patients indicates the skeletal maturity, this can be detected using biomarkers. And their detection locally, in saliva and gingival crevicular fluid (GCF), indicates the advancement of orthodontically induced alveolar bone remodeling [4]. Many research studies have been executed, suggestive of presence of array of molecules

indicating the skeletal growth turnover. The assessment of molecular biomarkers of bone remodeling in the body fluids such as saliva, GCF etc., would guide the clinicians to arrive at a better treatment plan for orthodontic therapy at the ideal time and estimate the advent of the treatment [4, 5].

Saliva is considered a medium for the microbes and transport, which is affected by status of oral health and the quantity and types of bacteria present in the oral cavity. It is also comprising of innate immune factors and various salivary defense proteins. The fixed orthodontic therapy induces site-specific bone resorption and formation and cytokines which are released from periodontal ligament (PDL) cells.

The complex combination of serum, host inflammatory cells, structural cells of oral bacteria and the periodontium leads to the formation of the GCF which arises from the plexus of gingival blood vessels in the gingival corium, lying underneath to the epithelial lining of the dentogingival space. It can be isolated from healthy sulcus as well [3, 5].

The origination of GCF components is from blood, subgingival plaque and host tissues. Presence of the transudate of the gingival interstitial fluid due to the osmotic gradient in the healthy periodontium is observed. There will be a steady increase in the volume with inflammation and greater capillary permeability [6]. Previously GCF was known as continuous transudate but currently it is considered as inflammatory transudate [7]. GCF comprises of host-derived substances which includes cytokines, antibodies, tissue degradation products and enzymes [8]. The inflammatory exudate increases by more than 5-fold during the inflammatory conditions, such as periodontal disease and gingivitis [9].

Orthodontic forces result in a condition which can be described as a consequence of the orthodontic force, a condition persists that involves a series of inflammation and repair intended at converting it into normal tissues and [10] according to some reports GCF reflects the immune reactions arising from both orthodontic force application and periodontitis [11, 12].

Nowadays many biomarkers are detected using saliva. It was recently discovered that several new isoforms for Nerve Growth Factor (NGF), Brain derived Neurotropic Factor (BDNF) and Calcitonin gene-related peptide (CGRP) were found in the saliva [3]. Identification of these isoforms can be utilized to develop subtle ways that can be considered to be methods to detect and analyze markers related to pain. The nuclear factor kappa B ligand and of the nuclear factor kappa B/osteoprotegerin (RANK/RANKL/OPG) signaling pathway being one of the several key factors that initiated the commencement of osteoclasts. The recruitment, differentiation and survival of osteoclasts are facilitated by the osteoblasts [12] which secretes a molecular biomarker RANKL. Induction of differentiation of immature osteoclasts into functional cells are due to the binding of RANKL with RANK (expressed at the surface of the osteoclast). Osteoblasts produce OPG which acts as a soluble receptor for RANKL. This inhibits the terminal stages of osteoclast differentiation [12].

Pain and discomfort are inevitable during orthodontic treatment [13]. Conventionally, the degree of pain is assessed subjectively using many pain scales [14]. Assessing pain objectively using salivary physiological biomarkers would benefit the clinician for appropriate pain diagnosis and management [13, 15]. The role of saliva in the diagnostic and prognostics is side-lined due to unavailability of a standardized collection procedure, even though well accepted by patients [3].

The neuropeptide, NGF protects the neurons and regenerates them. It plays an important role in hyperalgesia and its concentration increases during inflammation which is up regulated in response to noxious stimuli [16]. The occurrence and

development of pain and hyperalgesia are credited to be due to the role of CGRP and BDNF. CGRP and BDNF has been implicated in migraine and headache based on increased saliva and plasma concentrations during active pain periods [16, 17].

Only a few studies have investigated the levels of these above-mentioned neuropeptides in saliva [16–18]. Several patients describe much longer periods of pain and discomfort which are common during the first 1 or 2 days of the orthodontic treatment. Scheurer et al. reported that even after 7 days of insertion of a fixed appliance, 25% of all investigated patients still reported pain [19]. According to measurements at 4 h and 24 h, the intensity of pain generally increases with time, but falls to normal levels after 7 days of the orthodontic treatment [19]. Biomarkers can be used to characterize the sequential events following OTM. The rate, amount, and the activity of the released substances/biomarkers indicates the activity of individual cells and the metabolic activity involved in the tissues and organs [1]. These potential biological markers can be collected from different tissue samples. The sampling is done as per the required biomarker and the biological processes to be studied [1, 20]. Several possible biomarkers representing many of these biological changes during specific phenomenon like pain experienced during orthodontic treatment pain, bone remodeling, inflammation and root resorption have also been proposed [20]. The clinical application can be developed from the knowledge of biomarkers that can accelerate the orthodontic treatment as well.

2. Phases of orthodontic tooth movement (OTM)

There are two types of tooth movement namely: OTM and Physiological tooth movement. The physiological tooth movement occurs slowly in the cancellous bone in buccal direction or the cortical bone [21]. In contrast, OTM can occur both rapidly or slowly, it depends on the rate, physical characteristics and amount of the force application and the biological response of the Periodontal Ligament (PDL) [22]. The orthodontic force application can change the dental and paradental tissues, including the PDL, alveolar bone, dental pulp and gingiva resulting in pressure and tension sites at the tooth region [23].

Perinetti et al. [24] through their research study state that, one bone remodeling cycle involves four main phases namely: activation, bone resorption, reversal, and bone formation. Recent studies have exhibited that several enzymes are expressed during these phases which have been designated as biomarkers during bone remodeling namely Tartrate-resistant Acid Phosphatase (TRAP), Alkaline Phosphatase (ALP), Lactate Dehydrogenase (LDH), Aspartate Aminotransferase (AST), and many more.

Orthodontic therapy involves the supervision, guidance and correction of growing and maturing dentofacial structures which is based on the principle that if the teeth is subjected to prolonged pressure, consequently it will lead to remodeling of the bone. This OTM is exemplified by the remodeling of dental and paradental tissues [21].

In 1962, Burstone [25] stated the three phases of tooth movement, when rates of tooth movement are plotted against time:

1. Initial phase

2. Lag phase

3. Post-lag phase.

Phase I (initial)	24 h–2 days Initial tooth movement within the socket	Acute inflammatory response Vasodilation-migration of leucocytes-release of cytokines-cell signaling molecules (metabolic products of paradental remodeling)
Phase II (arrest)	20–30 days Movement stops	Chronic inflammation Continuation of migration of leucocytes Paradental remodeling
Phase III (acceleration)	40 days of accelerated tooth movement after initial force application	Another period of acute inflammation superimposing the on-going chronic inflammation
Phase IV (linear)	Overall tooth movement	Recruitment of macrophages, fibroblasts, osteoblasts, and osteoclasts Alkaline phosphatase activity

Table 1.
Phases of orthodontic tooth movement [27].

1. Initial phase: is described by immediate and quick movement which occurs in time period between 24 h to 48 h after the preliminary orthodontic force application to the tooth. This rate is largely recognized to the tooth movement in the PDL space.

2. Lag phase: The duration of this phase lasts up to 20–30 days which relatively shows little to no tooth movement. In this phase the region in which compression is applied, such region demonstrates the PDL hyalinisation. No subsequent tooth displacement occurs until the cells remove the necrotic tissues completely.

3. Post-lag phase: follows the lag phase, where the rate of tooth movement increases [26].

Pilon et al. [27, 28] divided the curve of tooth movement into four phases (**Table 1**). The GCF of tooth movement contain the biomarkers that indicate these phases and signaling pathways. Considerable increased levels of concentrations of cytokines responsible for inflammation and prostaglandins are observed.

The tissue changes that are involved during OTM includes compression region (which involves osteoblasts), tension region (which involves osteoclasts), pulp tissues and dental root [29]. Several possible biological factors or biomarkers representing these biological changes during particular phenomenon that is, bone remodeling. Inflammation and root resorption, have been identified. Similarly, lactic acid dehydrogenase (LDH) and dentin sialophosphoprotien (DSPP) are also potentially observed. A research study suggests that using sampling from four different sampling procedures, that is, saliva, GCF, tissue (biopsy), and serum, the biomarkers indicative of the ongoing biological processes can be identified [29]. The suggested amount and concentration of biomarkers during OTM are the best and practical sampling or testing procedure indicative of the biological phenomenon. The amount of precise force application and duration that should be used for each tooth during OTM can be decided based on the knowledge of these biomarkers. Ultimately, it produces an optimal treatment with mild side effects or accelerate the treatment [29].

3. Saliva and biomarkers

Most of the laboratory diagnostic procedures involves the analysis of the cellular and biochemical constituents of the blood. Saliva can be used in the diagnosis of

several diseases. This can be feasible and valuable for children and older adults due to the ease of collection of the fluid [30]. Saliva can be classified into two types: gland-specific saliva and whole saliva. It is feasible to collect saliva specific to different glands from individual salivary glands namely: parotid, submandibular, sublingual and minor salivary glands. Since the secretions from both the submandibular and sublingual salivary glands enter the oral cavity only through single duct known as Wharton's duct [31], hence collection of the saliva from submandibular and sublingual glands separately is challenging.

Orthodontists generally aims to gain ideal orthognathic conditions with fewer treatment times i.e.; shorter treatment time with longer treatment intervals with fewer appointments [32]. Conversely, when heavier force is applied to accelerate tooth movement, the oxygen tension in the periodontium will be conceded due to reduced vascular supply [33]. This will risk or expose the healthy supporting alveolar bone and periodontal structure leading to the slow progress of the treatment. In order to supervise the orthodontic tooth movement in a non-invasive approach in human beings, the alterations that appear during the examination of the profile and levels of various cytokines, enzymes, growth factors, and proteoglycans in saliva and GCF. Evidences support the elevated levels of several biomarkers or cytokines, that is, interleukin (IL)-1β, IL-6, epidermal growth factor (EGF), prostaglandin (PG) and proteoglycans, in the saliva and GCF [34–36]. Components of GCF namely ALP, TRAP, LDH and AST have been recognized to be potential biomarkers during OTM [37–40]. Study conducted by Shahrul et al. [41] showed that ALP, TRAP, and LDH also existed in saliva. Orthodontic treatment using a surface-enhanced laser desorption/ionization time of flight mass spectrometry (SELDI-TOF MS) approach the effects of the orthodontic treatment on salivary proteins has been performed as per the study by Zhang et al. [42]. The outcome of this approach determined the relatively low molecular weight proteins but not the identity of these proteins. Only the expression profile was cross-examined.

Saliva has protective and anti-microbial properties and contains a variety of growth factors [43, 44]. Saliva helps in the easy digestion of the food since it has lubricating functions [45]. The role of saliva and different salivary constituents responsible for its functions are summarized below in **Table 2** [30].

Salivary protein concentration ranges from 2 to 5 mg/mL which constitutes about 3% of the protein concentration of blood. Numerous proline-rich glycoproteins, immunoglobulin A and amylase are the major secretory proteins of the parotid glands, other antibacterial salivary proteins include lysozymes, peroxidases and lactoferrin. Submandibular and sublingual glands contribute mucous glycoproteins to oral fluid. Pathological analysis can be carried out using the saliva produced by specific glands. Since saliva contains constituents of other serum, whole saliva is used for the diagnosis of systemic diseases. The gingival fluid flows into the oral cavity. The constituents of gingival fluid can be derived from the local vasculature of the salivary glands. Analysis of saliva perhaps be useful for the diagnosis of different hereditary disorders, endocrine disorders, malignant, autoimmune and infectious diseases, as well as in the assessment of therapeutic levels of drugs and in the monitoring of illicit use of drugs.

Evaluation of the fluids from the individual salivary glands can help in detecting the infection and obstruction. Mixture of oral fluids can be present in the whole saliva or mixed. This may include the secretions from both the major and minor salivary glands including several constituents of non-salivary origin. The fluids of non-salivary origin may include expectorated bronchial and nasal secretions, GCF, serum and blood derivatives from oral wounds [46–49].

Functions salivary components involved:

1. Protective functions
   - Lubrication—Mucins, proline-rich glycoproteins, water.
   - Antimicrobial—Amylase, complement, defensins, lysozyme, lactoferrin, lactoperoxidase, mucins, cystatins, histatins, proline-rich glycoproteins, secretory IgA, secretory leukocyte protease inhibitor, statherin, thrombospondin.
   - Growth factors—Epidermal growth factor (EGF), Transforming growth factors (TGF-α), TGF-β, fibroblast growth factor (FGF), insulin-like growth factor (IGF-I & IGF-II), Nerve growth factor (NGF).
   - Mucosal integrity—Mucins, electrolytes, water.
   - Lavage/cleansing—Water.
   - Buffering—Bicarbonate, phosphate ions, proteins.
   - Remineralization—Calcium, phosphate, statherin, anionic proline-rich proteins.
2. Food- and speech-related functions:
   - Food preparation—Water, mucins.
   - Digestion—Amylases, lipase, ribonuclease, proteases, water, mucins.
   - Taste—Water, gustin.
   - Speech—Water, mucins.

Table 2.
The major functions of saliva [30].

Evaluation of the systemic disorders is done by the salivary analysis of the whole saliva collected with or without stimulation. There are two procedures to collect the saliva using stimulation, they are gustatory stimulation (i.e., application of citric acid on the subject's tongue [50]; or by masticatory action (i.e., from a subject chewing on paraffin). Constituents, pH and concentration of the fluid depends on the quantity of saliva collected by the stimulation method. Unstimulated saliva is collected without gustatory, masticatory, exogenous or mechanical stimulation. The factors that affect the salivary flow rate depends on the degree of hydration, olfactory stimulation, exposure to light, body positioning, seasonal and diurnal factors. There are two appropriate methods to collect the saliva, they are by the draining or drooling method, in which saliva can drip off the lower lip. In the second methods which is the spitting method the subject expectorates saliva into a test tube [51].

Specialized epithelial cells make up the salivary glands, based on their structure, these cells can be divided into two specific regions: the ductal and acinar regions. During the primary salivary secretion, the ductal cells actively absorb most of the Na^+ and Cl^- ions and secrete small amounts of K^+ and HCO_3^- and some proteins. This modifies the primary salivary secretion into a hypotonic final salivary secretion when it enters the oral cavity [52].

In the acinar region most of the protein synthesis and secretion takes place, this is where the oral fluid is generated as well. The amino acids enter the acinar cells through active transport. After the intracellular protein synthesis, secretory stimulation releases most proteins, these are stored in storage granules [53, 54]. Description of three models for acinar fluid secretion area are available, which include the active passage of anions into the lumen and passage of osmotic gradient water from the interstitial fluid to the salivary lumen [55, 56]. Fluid obtained at the initial stage is isotonic in nature. This is derived from the local vasculature whereas the acinar cells

are water-permeable, and the ductal cells are impermeable. The autonomic nervous system(sympathetic and parasympathetic system) controls the salivary secretion and its signaling mechanism involves the binding of neurotransmitter (primarily acetylcholine and norepinephrine) to plasma membrane receptors and signal transduction *via* guanine nucleotide-binding regulatory proteins (G-proteins) and activation of intracellular calcium signaling mechanisms [52, 57–59]. A few diagnostic uses of saliva like viral infections, including hepatitis and HIV, and in the detection of certain endocrine disorders have been demonstrated.

Certain markers in the serum cannot be relied upon but with the salivary samples, the levels of certain markers are consistent than its manifestation. The components of the biomarker molecules and normal saliva have similar physicochemical characteristics. Hence the diffusion of lipophilic molecules into the saliva is easier than the lipophobic molecules. The molecules involved in the biological processes reach the saliva through different mechanisms. Hence extraction of molecules using the methods such as ultrafiltration and active transport have also been proposed for many substances recently. Whereas previously passive diffusion was the most common mechanism for drugs and hormones.

For accurate diagnosis, an appropriate relationship must be established between the biomarker concentration in serum and its concentration in saliva. Normalcy in the salivary gland function is necessary for the collection of salivary molecules or cytokines with diagnostic value. The flow rate of the saliva and its concentration is expected to vary between individuals and in the same individual under various conditions. Erratic collection of the serum markers is possible in the whole saliva due to the oral wounds and through GCF flow. Effectiveness of such samples may be questionable as these parameters may interfere in the correct diagnosis based on the salivary constituents [60]. Apart from some of the systemic disorders, other factors like the medications and radiation will affect salivary gland function, the quantity and consequently the composition of saliva [61, 62]. The whole saliva may contain some proteolytic enzymes that come from the host and there may be presence of enzymes derived from the oral microorganisms [63]. These enzymes can affect the stability, consistency and reliability of certain diagnostic biomarkers. Degradation of some molecules happened during intracellular diffusion into saliva. The diagnostic functional value of the marker may be affected by any condition or medication.

Despite the limitations of saliva to be used for diagnostic purposes, it is becoming popular as per the evidences suggested by many recent researches. Commercially available salivary diagnostic tests are currently being used by patients, researchers, and clinicians. Objective and qualitative detection that is the detection of the presence or absence of a biomarker using saliva is possible but it is not a feasible option for the quantitative diagnosis. Saliva also plays a major role in eliciting and monitoring the hormone levels, especially steroids which facilitates repeated sampling in short time intervals, which may be particularly important for hormone monitoring and avoiding patient and clinician compliance problems.

Nevertheless, before a salivary diagnostic test can replace more conventional methods, the diagnostic values of a new salivary test must be compared with the gold-standard. The effectiveness of a new test must be determined in terms of specificity, sensitivity, reproducibility and the correlation with an established disease diagnostic criterion.

The known functions of proteins identified by proteomic analysis are summarized in **Table 3**. Using the proteomics approaches, these proteins have been identified previously [32, 64, 65]. There has been no reports of the changes in the protein

Protein	Known function
Protein S100-A9 (S100 calcium-binding protein A9) (Calgranulin-B)	i. Calcium-binding protein. ii. At the sites of wounding, it promotes phagocyte migration and infiltration of granulocytes. iii. Takes part as a proinflammatory mediator in acute and chronic inflammation.
Serum albumin precursor	i. It binds with water, $Ca2^{+}$, Na^{+}, K^{+}, fatty acids, hormones, bilirubin, and drugs. ii. The main function is to regulate the colloidal osmotic pressure of blood. iii. Major zinc transporter in plasma.
Immunoglobulin J chain	i. Links two monomer units of either IgM or IgA. ii. Helps in binding IgM or IgA to a secretory component.
Ig alpha-1 chain C region	i. A major immunoglobulin class in the body secretions. ii. Serves as a defence against local infection and prevents access of foreign antigens.
Cysteine-rich secretory protein 3 precursor (CRISP-3)	i. Innate immune response ii. Potential biological marker for prostate cancer
Hemoglobin subunit beta (Hemoglobin beta chain) (Beta-globin)	Role in the transportation of oxygen from the lung to the various peripheral tissues.
14-3-3 protein σ (Stratifin) (Epithelial cell marker protein 1)	i. An adapter protein. ii. Results in the modulation of the activity of the large number of binding partners.

Table 3.
Summary of identified proteins and their known functions [30].

expression in relation to the orthodontic treatment, tooth movement and its forces. The **Table 3** lists the functions of proteins and their predictive role in the OTM.

3.1 Protein S100-A9

During acute and chronic inflammation Protein S100-A9 (S100-A9) is a calcium-binding protein that functions as a proinflammatory mediator. It is found in high concentration in an inflamed tissue. Previous research described that S100-A9 was concerned in chondrocytic and osteoblastic maturation, matriceal calcification and was noticeable in osteoclasts [66]. Therefore, the presence of osteoclast indicates an active process of bone resorption. It is also been stated that it regulates cartilage destruction and joint inflammation during antigen-induced arthritis [67]. A recent study showed an obvious downregulation of S100-A9 protein, this indicates that there may not be an involvement of the protein during bone resorption in fixed orthodontic tooth movement. Its involvement may be in the inflammatory conditions as suggested by the data from the previous study. As per the study, on day 14 of the treatment, the inflammatory process was not active. The orthodontic treatment for 14 days resulted in the downregulation of this above protein indicated by the suppression of inflammation.

3.2 Immunoglobulin J chain (IgJ)

Immunoglobulin J chain (IgJ) is a constituent of IgA or IgM, whereas Ig Alpha-1 chain C region (IgAC) is a major immunoglobulin class in the body secretions.

Both are common elements of the immune response in humans. Acute inflammatory responses were noticed in the periodontal tissues surrounding the mechanically stressed teeth, in the early phase of the OTM [24]. Recent study also exhibited an obvious downregulation of both IgAC and IgI after 14 days of the fixed orthodontic therapy. Previous study also did not show any increase of LDH after 14 days of orthodontic activation [41]. After these studies researchers suggested that no further inflammation had occurred at that period.

3.3 Cysteine-rich secretory protein 3 precursor (CRISP-3)

CRISP-3 shows the presence of the exocrine secretion and secretory granules of neutrophil in them. It has notable functions in innate immunity [68] and it is a potential biological marker for prostate cancer [69]. According to the results from the recent study after 14 days of orthodontic treatment, the presence of this protein was noticed. There is not much clarity regarding its relationship with orthodontic tooth movement.

3.4 Serum albumin precursor (ALB)

ALB and hemoglobin subunit beta (HBB) are serum proteins. These serum proteins are responsible for the increase in subjects with periodontal disease [70]. ALB is considered as a major zinc transporter in the plasma which regulates the colloidal osmotic pressure of blood [71]. The HBB is a subunit of hemoglobin containing two beta units and four subunits with two alpha. Transport of oxygen from the lung to different peripheral tissues involves the alpha and beta subunit carrying an ion-containing molecule (heme) in each of them [72]. HBB is commonly found in the red blood cells (RBCs) but its function(s) in saliva is still unknown. Recent study showed that ALB was present only at day 0 of treatment. And its role(s) in orthodontic tooth movement is also still unclear.

3.5 Protein: 14-3-3 σ

On Day 0, an adaptor protein—14-3-3 σ also known as epithelial cell marker protein 1 or stratafin (SFN) was found to be present. This binds to many partners and results in the modulation of the activity of the binding partner(s). In several types of human cancers, loss of protein 14-3-3 σ expression has been observed suggesting its role as a tumor suppressor protein [73]. However, there is lack of evidence on the role of SFN protein in inflammation or bone resorption and formation, and the role(s) it may play during fixed orthodontic tooth movement.

Orthodontic and dentofacial orthopedic therapeutic appliances used in the treatment and correction of various maxillofacial and dento-maxillary anomalies with skeletal and dental problems most frequently believe that the orthodontic force application of high intensity can induce a localized inflammatory process around the tooth and tooth supporting structures. Due to the presence of this inflammatory process there is an increased synthesis of free radicals secondarily produced which is followed by the oxidative stress [74]. In the literature till date, there are very limited number of human studies and evidences on the oxidative stress and oxidative damage that may occur due to an aseptic inflammation in tissues caused because of orthodontic tooth movements [74].

A recent study performed in 2009 by Olteanu et al. [60] compared and determined the amount of oxidative stress markers in the saliva of patients, they were treated using orthodontic appliances for a predefined stipulated time period. The time periods that were predefined for determination were: before and after 1 h of treatment, 24 h and 7 days after the initiation of the treatment. At 24 h, maximum variation was observed in the concentrations of the saliva markers of the oxidative stress for ceruloplasmin and malondialdehyde (MDA). And for the hydrogen donors at one hour respectively, and at the 7 days from the placing of an appliance, the concentrations of markers were similar to the values observed in the initial phase of the tests. Concentration of saliva markers for oxidative stress showed changes but this cannot prove pathological processes prevalent in the patients with orthodontic appliances at the level of the oral cavity.

Recently, the research study performed using saliva by Ozcan et al. [75] had the objective to evaluate the changes in some oxidative stress markers for the determining of oxidative stress damage that occurs in the process of bone and tissue remodeling, including dysfunction of periodontal tissue caused by orthodontic tooth movement. At certain time intervals, the unstimulated saliva samples of patients with fixed orthodontic appliances were collected. The time intervals followed were: just before treatment, at the 1st month of treatment and at the 6th month of treatment. Investigations were conducted using spectrophotometric method to detect nitric oxide (NO) and MDA. The TNF-α, IL-1β, and 8-OHdG levels were detected using ELISA method. In the results, at any of the predefined time period, the study did not show any significant change in the saliva in all biochemical parameters. In another study involving the unstimulated saliva samples of individuals with fixed orthodontic appliances, there were no significant differences observed when compared to the control group in kynurenine concentration [76]. And the data presented in the study at least at the first 6 months of the treatment indicate that orthodontic materials and orthodontic tooth movement used in orthodontic treatment do not cause oxidative damage in the oral cavity.

4. Pain and tooth movement

Nowadays many biomarkers are detected using saliva. It was recently discovered that several new isoforms for Nerve Growth Factor (NGF), Brain derived Neurotropic Factor (BDNF) and Calcitonin gene-related peptide (CGRP) were found in the saliva [3]. Identification of these isoforms can be utilized to develop subtle ways that can be considered to be methods to detect and analyze markers related to pain.

Pain and discomfort are inevitable during orthodontic treatment [6]. Conventionally, the degree of pain is assessed subjectively using many pain scales [7]. Assessing pain objectively using salivary physiological biomarkers would benefit the clinician for appropriate pain diagnosis and management [6, 8].

These potential biological markers can be collected from different tissue samples. The sampling is done as per the required biomarker and the biological processes to be studied [1, 9]. Several possible biomarkers representing many of these biological changes during specific phenomenon like pain experienced during orthodontic treatment pain, bone remodeling, inflammation and root resorption have also been proposed [20]. The clinical application can be developed from the knowledge of biomarkers that can accelerate the orthodontic treatment as well.

Development of objective markers of nociception and pain helps in diagnosis of pain and its management. Standardization of pain assessment objectively is important to avoid bias in research studies. Tools that are sensitive and specific to pain fulfilling certain criteria's like being observer-independent, not reliant on the patient's ability to communicate and not influenced by disease characteristics are needed in developing an objective method of pain assessment [13]. A review by Cowen et al. [13] states that the objective biomarkers of nociception or pain which have been validated for clinical use, although there are currently promising strategies like monitoring changes in the autonomic nervous system, biopotentials, neuroimaging and composite algorithms. There is a serious need for theoretically promising and clinically useful objective marker for assessment of pain. Restricted use of physiological markers as 'objective' measures of pain and nociception is due to the lack of evidence in support of its use as biomarker. Biomarker research in saliva for pain and nociception as part of clinical phenotyping should be watched closely.

Fleming et al. [14] suggests that unlike the bracket type, the subjective pain experience at 4 h, 24 h, 3 days, and 7 days following fixed orthodontic appliance placement [14]. The subjective pain was recorded using Visual Analog Scale (VAS) [14].

In cross sectional study conducted by Jasim et al. [3], the levels of nerve NGF, CGRP and BDNF were determined using novel western blotting-based technology using Capillary Isoelectric Focussing (CIEF) Immunoassay. Glutamate and substance P (SP) was determined using ELISA. Numerous new isoforms were found for NGF, CGRP and BDNF in saliva. In expression and chemiluminescence levels, the isoform pattern showed significant difference between different collection methods. In this study, new sensitive methods to study pain related markers in saliva were developed. And this study was the first to:

1. detect NGF, CGRP, BDNF, Glutamate and S P (SP) in five different salivary types,

2. to develop a new protocol/method for analysis of different isoforms of NGF, CGRP and BDNF,

3. show quantifiable levels of several isoforms of NGF, CGRP and BDNF in human saliva, and finally.

4. establish a correlation between the glutamate level in stimulated whole saliva and plasma.

Activation of the orthodontic appliance induces painful sensations due to the inflammatory process, this occurs as part of the tooth movement related to tissue remodeling. It is established that the immunoreactive neuron C-fos is involved in the transmission of nociceptive information expressed bilaterally in the lateral parabranchial nucleus. And ipsilaterally in the trigeminal subnucleus caudalis past the initial 24 h of orthodontic force application. Similarly, fos-like immunoreactive neurons were distributed in other brain regions such as the neocortex, thalamic nucleus and dorsal raphe [77]. Nociceptive information by tooth movement is modulated and transmitted in several regions of the brain. Endogenous pain control systems are activated by these stimuli, including descending monoaminergic pathways [78].

Initial studies suggested that through dopaminergic and serotonergic systems, the nociception is nociception is regulated [77]. Subsequently, another experiment

performed showed an increase in serotonin turnover in the medulla, indicating the bulbospinal serotonergic pathway activation by nociceptive neurological response [78]. Therefore, an indirect nociceptive mechanism operating during tooth movement occurs that suggests a continuous and delayed nociceptive response, which is expected to regulate the masticatory function during active tooth movement.

A recent case report published data on administration of MK-801 in rats (a non-competitive antagonist of N-methyl-Daspartate receptors), intraperitonially before tooth movement. The results suggested the N-methyl-D-aspartate receptors blockade along with neuronal suppression of sensory nuclear complex of the trigeminal nerve branch. Subsequently, these effects were found to increase the neuronal activity in the descending antinociceptive system, including dorsal raphe nucleus, nuclear raphe magnus, ventrolateral PAG, and Edinger-Westphal nucleus. Following, during orthodontic tooth movement, these results indicated a pharmacological way to decrease pain perception [79].

Salivary biomarkers as a measure has the potential to be an objective approach and a diagnostic tool for the studies related to pain. However, there is a need of estimating the different collection methods and develop more profound techniques for analysis. These biomarkers have a crucial part in objectively assessing the pain.

5. Gingival crevicular fluid (GCF) and biomarkers

GCF is an exudate that can be collected from the gingival sulcus in periodontium, which provides a prospective source of factors or biomarkers associated with the changes and destruction in the underlying periodontium that generally occurs during the orthodontic force application during fixed orthodontic treatment [80].

Due to its non-invasive nature and ease of repetitive sampling from the same site with the help of filter paper strips, gingival washings, platinum loops and micropipettes, GCF is commonly collected for the examination and check the levels and concentration of these biomarkers during the orthodontic force application. This fluid is easily available as that of saliva in the oral cavity and is usually used to analyze various biochemical markers [80].

The importance of GCF biomarkers in periodontal effects is tabulated in **Table 4**.

GCF which can be described as a transudate or an exudate arises at the gingival margin where its flow rate is of 0.05–0.20/min which indicates gingival inflammation [81]. Various biochemical markers such as prostaglandin production and the action of various extracellular and intracellular factors, such as IL-6, IL-1, TNF, epidermal growth factors, cathepsin, aspartate aminotransferease, microglobulin, alkaline phosphatase, and lactate dehydrogenase are analysed by this GCF.

Various cell mediators or enzymes are produced due to the remodeling changes in the PDL and the alveolar bone that can be used as the biomarkers of orthodontic treatment [66, 82]. The initial works conducted by Embery, Waddington [83] and Last et al. [84], proved the presence of many proteoglycan, tissue proteins and GAGs in GCF and also reported presence of chondroitin-4-sulphate in GCF from the pressure side of tooth movement. Biological alteration is caused in deep-seated tissues due to that the increase in chondroitin-4-sulphate since the orthodontic model is a nonplaque and non-disease-related process.

Uematsu et al. [85, 86] found elevated levels of IL-1, IL-6, TNF, epidermal growth factors, TGF 2 and microglobulin during the orthodontic treatment in the GCF. Lee et al. [87] and Grieve et al. [88] also reported stated the similar finding for IL-1, and

Inflammatory mediators
• Protein E2 (P E2) -bone resorption
• Substance P (S P) (neuropeptide)-bone resorption
• Epidermal growth factor (EGF)-bone resorption
• Transforming growth factor (TGF)-bone remodeling
• RANKL - stimulation of osteoclastic differentiation
• Osteoprotegerin-inhibition of osteoclastic differentiation
• Granulocyte macrophage colony stimulating factor-bone turn over
• α2 microglobulin-enhancer of IGF-1
• IL 1β, 2, 6, 8-bone remodeling
• Myeloperoxidase–enzyme in PMN-inflammation
Metabolic products of paradental remodeling
• Hyaluronic acid (GAG)-indicator of breakdown of gingival tissue
• Chondroitin sulfate (GAG)-indicator of breakdown of alveolar bone and
• PDL
• Pentaxrin-3 (TNF stimulated gene14)-marker of inflammation
• Osteocalcin-bone turnover
• Insulin growth factor-regulators of cell differentiation and apoptosis
• Pyridinoline, deoxypyridinoline-indicators of bone metabolism
• N-telopeptide-bone resorption
• Dentin matrix protein-root resorption
Enzymes
• Acid phosphatase- bone resorption
• Alkaline phosphatase-bone formation
• Aspartate amino transferase-cell necrosis
• CathepsinB-extracellular matrix degradation
• Matrix metalloproteins (1, 2 and 8)-breakdown denatured collagen
• β glucuronidase-marker of granule release by PMN
• Lactate dehydrogenase-indicator of cell death
GCF, Gingival crevicular fluid; IGF-1, Insulin-like growth factor-1; GAG, Glycosaminoglycans; PMN, Polymorphonuclear neutrophil; TNF, Tumor necrosis factor; PDL, Periodontal ligament.

Table 4.
List of GCF biomarkers and their role in orthodontic tooth movement.

PGE2. Lowney et al. [89] for TNF. Griffiths et al. [90] demonstrated the presence of osteocalcin in GCF from teeth which is subjected to the orthodontic treatment force application. A recent study by Insoft et al. [91] also found an increased level of alkaline phosphatase during the first 3 weeks of orthodontic treatment, whereas acid phosphatase increased in successive weeks. Perinetti et al. [92] also determined the aspartate aminotransferase along with the alkaline phosphatase activity in GCF. Orthodontic force application induced an increase in the lactate dehydrogense activity in GCF as per a recent study by Serra et al. [93]. After the study it is proposed to be a sensitive factor or biomarker for periodontal metabolism. Sugiyama et al. [82] suggested that cathepsin B involved in ECM degradation and reported its increase in the amount in GCF.

After an orthodontic force presentation for 4–8 h Apajalahti et al. [94] found a significantly higher amount of MMP-8 in GCF. They suggested that MMP-8 reflects the enhanced periodontal remodeling, this is the effect of increased expression and activation of GCF. From the studies, it was concluded that presence of such markers in GCF during the orthodontic studies are useful in identifying the bone-remodeling activities. Therefore, GCF can be considered a promising topic for future research, as these investigations have already begun to provide an insight into the progressive aspects of remodeling.

According to the QUOROM statement suggestions, a systematic review was conducted by Allgayer et al. [95] in 2014 by strictly adhering to the guidelines suggested by PROSPERO [95]. Several key databases namely PubMed, Embase, Cochrane library, MEDLINE, and Web of Science were searched in May 2014 using the MESH terms 'Orthodontics, Corrective', 'IL-17', or 'helper 17 cell', or 'helper T Cells', or 'TH 17', 'IL 17', 'IL-23', 'crevicular fluid', 'IL 23', and using the free text terms 'GCF, gingival crevicular fluid, regulatory proteins, tooth displacement, cytokines, inflammatory factors, root resorption and canine distalization', and, by reference tracking an additional search was performed. The search results from each database were compiled, combined and the duplicate results were eliminated (**Table 5**).

The results of this systemic review provide an insight by identifying the 115 potentially relevant studies. **Table 5** below represents an overview of the outcomes. Among these studies, further analysis of the titles, abstracts, and full texts revealed that for this systematic review the major 25 studies were relevant [95]. Many studies were performed on mixed samples of young adults and adolescents, and two related studies reported the levels of GCF cytokines in different age groups (**Table 5**). More than 20 subjects were found to be recruited for only 3 studies. Out of 25 studies, seventeen of them used the maxillary canine as their study tooth, and the orthodontic force system was distalization of the canine with either continuous arch wires or sectional wires. The other eight studies addressed insertion of separation elastics (two), Hyrax appliance (two), aligning movement (three), and cervical headgear (one).

MMPs are considered to be the main endogenous chemical mediators of the pathologic destruction of tissues in periodontitis [96]. Extensive research studies have been performed to assess the levels of MMPs in GCF [97] and saliva and also, they have been found to be elevated in patients suffering with periodontitis compared to subjects with healthy periodontium. Additionally, after the periodontal therapy there was drastic decrease in the levels of MMPs in GCF. The levels of MMP-8, 9 MMP-3,10 and MMP-13,11,12 have been linked with the periodontal disease progression in GCF. MMPs also play a dominant role in the remodeling process of PDL during orthodontic tooth movement.

A study in dogs by Redlich et al. [96, 98] suggests an increase in the activity of MMP-1 and mRNA levels in the compression side of the gingiva during orthodontic tooth movement. Similarly, a study performed on rats also demonstrates an increased expression of MMP-13, mRNA, and MMP-8 in the PDL during active tooth movement [13]. Using MMP inhibitors orthodontic tooth movement can be prevented or delayed in mice and rats [96, 98]. A few studies performed on humans [96] have enumerated the presence of MMPs in GCF during orthodontic tooth movement and have stated the alterations in their levels during the orthodontic force application. Additionally, in orthodontic patients treated with fixed appliances, the total collagenase activity in the GCF has been revealed to be 10-fold that of control GCF [99].

Surprisingly, it is found that the effects of orthodontic force application on teeth affected by periodontal disease has not been significantly studied. However, a few

Reference/article	Sample (n) Age (yr)	Sampling (points)	Method	Cytokine	Peak	Results
Alikhani et al 2014	20 19.5-33.1	0, 24 h, 28 d	Canine distalization	IL-1β, IL-1α, IL-6, IL- 8, TNF, MCP-1		Tend to increase
Enhos et al 2014	20 18+-3	0, 24, 48, 168 h, 30 d	Canine distalization	RANKL OPG		OPG and RANKL levels vary as a result of force application
Gastel et al 2011	24 14+-1	0, 365 d	Headgear	IL-2 IL-4 IL-6 IL-8 IL-10 GM-CSF INF TNF MCP-1 IP-10		No significant alteration No significant alteration Tend to increase Tend to increase No significant alteration No significant alteration No significant alteration No significant alteration No significant alteration No significant alteration
Tzannetou 2008	9 10-18	0, 24 h, 7 d, 14 d, 21 d	Hyrax RME	IL-1β		Increased during active RME; remained high in retention phase
Karacay et al 2007	10 15+-1	0, 1, 24, 168 h	Canine distalization	TNF-α	1 d	Hybrid: no change with time Distraction: higher than hybrid
Iwasaki 2006	10 15+-4	0, 1, 3, 7, 14, 28, 42, 56, 70, 84 d	Canine distalization	IL-1β IL-1ra		Tooth movement rate relate to IL-1 gene polymorphisms
Dudic 2006	18 9-14	-7 d, 0, 1 min, 1 h, 1 d, 7 d	Separation elastics	IL-1β PGE2 SP	1 d 1 d 1 d	Higher levels at tension sites Higher levels at tension sites
Basaran 2006	18 16-19	0, 7 d, 21 d, 6 mns, +7 d, +21 d	Canine distalization	IL-1β TNF-α		Tend to increase Tend to increase

Reference/article	Sample (n) Age (yr)	Sampling (points)	Method	Cytokine	Peak	Results
Kawasaki 2006	15 15+-3 15 31+-4	0, 1, 24, 168 h	Canine distalization	RANKL OPG	1 d 1 d	Lower ratio in adults Lower ratio in adults
Nishijima 2006	10 15+-2	0, 1, 24, 168 h	Canine distalization	RANKL OPG	1 d 1 d	No change at 1 h or 168 h No change at 1 h or 168 h
Basaran 2006	17 17+-2	0, 7 d, 21 d, 6 mns, +7 d, +21 d	Canine distalization	IL-2 IL-6 IL-8		Tend to increase at 7 d, back at 21 d Tend to increase at 7 d, back at 21 d Decrease at 7 d, back to baseline at 21 d
Giannopoulou 2006	18 9-14	0, 1, 24, 168 h	Separation elastics	IL-1β PGE2	1 d	Associated with pain at 24 h Weak association with pain at 1 h
Yamaguchi 2006	9 22-2	0, 1, 4, 8, 24, 72, 120, 168 h	Canine distalization	IL-1β	1 d	Correlated with P-substance
Toia 2005	6 10-13	0, 4 h, 10 d	Aligning	IGF	4 h	Decreased 10 d after time-dependent decrease
Hoshino-Itoh 2005	10 23+-3	0, 1, 24, 168 h	Canine distalization	PAI	1 d	Increased only at 24 h Increased only at 24 h
Iwasaki 2005	10 10-30	-28, -14, 0, 1, 3, 14, 28, 42, 56, 70, 84 d	Canine distalization	IL-1β IL-1ra		The ratio of both correlate with the rate of tooth movement
Tuncer 2005	10 15-17	0, 1, 24 h, 6 d, 10 d, 30 d	Canine distalization	IL-8		Tend to increase in early phase
Lee 2004	10 18-22	0, 1, 24, 168 h	Canine distalization	IL-1β PGE2	1 d 1 d	Continuous: 168 h back to baseline Interrupted: higher level at reactivation Continuous: 168 h back to baseline Interrupted: remained high for 1 wk

Reference/article	Sample (n) Age (yr)	Sampling (points)	Method	Cytokine	Peak	Results
Ren 2002	43 10-14 41 21-27	0, 24 h	Aligning	IL-6 PGE2		Upregulated in both age groups Increased only in youngsters Increased only in adults
Iwasaki 2001	7 12-16	-28, -14, 0, 1, 3, 14, 28, 42, 56, 70, 84 d	Canine distalization	IL-1β IL-1ra		28 d periodic changes, no site difference No periodicity, no site difference
Tzannetou 1999	9 10-18	0, 24 h, 7 d, 14 d, 21 d	Hyrax RME	IL-1β		Increased during active RME; remained high in retention phase
Uematsu 1996	12 14	0, 1, 24, 168 h	Canine distalization	IL-1β IL-6 TNF-α	1 d 1 d 1 d	No change at 1 h or 168 h No change at 1 h or 168 h No change at 1 h or 168 h No change at 1 h or 168 h
Uematsu 1996	12 14	0, 1, 24, 168 h	Canine distalization	TGF-β1	1 d	No change at 1 h or 168 h
Lowney 1995	20 12-36	0, 5 min	Canine distalization	TNF-α		More than twofold increase
Grieve 1994	10 24-27	0, 1, 24, 48, 168 h	Aligning	TNF-α PGE	1 d 1 d	Increased at 1 h, back to baseline at 168 h Stay high at 48 h, back to baseline at 168 h

Table 5.
A brief insight on those studies assessing biomarkers or the cytokines in gingival crevicular fluid (GCF) in orthodontics.

retrospective and longitudinal studies [97, 99] indicated that the orthodontic force application can be tolerated by periodontally compromised teeth without any additional damage to the periodontium.

6. Conclusion

Present dissertation deals with brief insight into the biomarkers, connected to orthodontic treatment and progression. Suitable biomarkers may be used to distinguish the sequence of events following OTM. Biophysical mechanisms are involved in the displacement of tooth in the periodontal space and shift of stimulus from continuous force application. The assessment of these biological mechanisms can be done by the evaluation of rate and amount of synthesis of biomarkers in periodontium. This knowledge of the ongoing process occurring in periodontal tissues during orthodontic and dentofacial orthopedic therapies in turn may help us to make proper choice of mechanical orthodontic loading, the amount of orthodontic force application during the treatment and period of treatment may be shortened. It also helps to avoid adverse consequences such as bone loss or root resorption associated with orthodontic treatment. The essential goal is to develop a screen test based on these factors or biological markers that could be used non-invasively and easily by the orthodontist at chairside to monitor the ongoing process and detect early root resorption. Several sensitive, salivary biomarkers are available to detect the biomechanical changes occurring during orthodontic tooth movement and pain occurring during fixed orthodontic therapy. Further focussed research might help to analyze the sensitivity and reliability of these biomarkers (cytokines), which in turn can lead to the development of chairside tests to assess the pain experienced by patients during orthodontic therapy and finally the outcome of the fixed orthodontic therapy. There is an enormous scope for research in this field which may be a boon for future orthodontic treatment and modalities.

Conflict of interest

None.

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References

[1] Nagarajan D, Usha K, Rao GU, Vijjayakanth M, Jayanthy M, Sabarigirinathan C, Sriramaprabu G, Vinayagavel K, Amit K. Biomarkers in Orthodontics. Int J Oral Health Med Res 2015;2(3):88-90.

[2] Karacay S, Saygun I, Bengi AO, Serdar M. Tumor Necrosis Factor–α Levels during Two Different Canine Distalization Techniques. Angle Orthod. 2007 Jan;77(1):142-147.

[3] Jasim H, Carlsson A, Hedenberg-Magnusson B, Ghafouri B, Ernberg M. Saliva as a medium to detect and measure biomarkers related to pain. Scientific reports. 2018 Feb 19;8(1):3220.

[4] Taba M, Kinney J, Kim AS, Giannobile WV. Diagnostic biomarkers for oral and periodontal diseases. Dental Clinics. 2005 Jul 1;49(3):551-571.

[5] Alhadlaq AM, Patil S. Biomarkers of orthodontic tooth movement in gingival crevicular fluid: a systematic review. J Contemp Dent Pract. 2015 Jul 1;16(7):578-587.

[6] Yamaguchi M. RANK/RANKL/OPG during orthodontic tooth movement. Orthodontics & craniofacial research. 2009 May;12(2):113-119.

[7] Delima AJ, Van Dyke TE. Origin and function of the cellular components in gingival crevice fluid. Periodontology 2000. 2003 Feb;31(1):55-76.

[8] Cimasoni G. Method of collection. Crevicular fluid updated. 1983 Basel.

[9] Kowashi Y, Jaccard F, Cimasoni G. Sulcular polymorphonuclear leucocytes and gingival exudate during experimental gingivitis in man. Journal of periodontal research. 1980 Apr;15(2):151-158.

[10] Meikle MC. The tissue, cellular, and molecular regulation of orthodontic tooth movement: 100 years after Carl Sandstedt. Eur J Orthod. 2006 Jun 1;28(3):221-240.

[11] Ren Y, Hazemeijer H, de Haan B, Qu N, de Vos P. Cytokine profiles in crevicular fluid during orthodontic tooth movement of short and long durations. J Perio. 2007 Mar 1;78(3):453-458.

[12] Ren Y, Maltha JC, Van′ t Hof MA, Von den Hoff JW, Kuijpers-Jagtman AM, Zhang D. Cytokine levels in crevicular fluid are less responsive to orthodontic force in adults than in juveniles. Journal of clinical periodontology. 2002 Aug;29(8):757-762.

[13] Cowen R, Stasiowska MK, Laycock H, Bantel C. Assessing pain objectively: the use of physiological markers. Anaesthesia. 2015 Jul;70(7): 828-847.

[14] Fleming PS, Dibiase AT, Sarri G, Lee RT. Pain experience during initial alignment with a self-ligating and a conventional fixed orthodontic appliance system: a randomized controlled clinical trial. Angle Orthod. 2009 Jan;79(1): 46-50.

[15] Hawker GA, Mian S, Kendzerska T, French M. Measures of adult pain: Visual Analog Scale for Pain (VAS Pain), Numeric Rating Scale for Pain (NRS Pain), McGill Pain Questionnaire (MPQ), Short-Form McGill Pain Questionnaire (SF-MPQ), Chronic Pain Grade Scale (CPGS), Short Form-36 Bodily Pain Scale (SF-36 BPS), and Measure of Intermittent and Constant

Osteoarthritis Pain (ICOAP). Arthritis Care & Research. 2011;63(S11): S240-S252.

[16] Jang, M.-U., Park, J.-W., Kho, H.-S., Chung, S.-C., & Chung, J.-W. (2010). Plasma and saliva levels of nerve growth factor and neuropeptides in chronic migraine patients. Oral Diseases, 17(2), 187-193.

[17] Zidverc-Trajkovic J, Stanimirovic D, Obrenovic R, Tajti J, Vécsei L, Gardi J, Németh J, Mijajlovic M, Sternic N, Jankovic L. Calcitonin gene-related peptide levels in saliva of patients with burning mouth syndrome. J Oral Path Med. 2009 Jan;38(1):29-33.

[18] Mandel AL, Ozdener H, Utermohlen V. Identification of pro-and mature brain-derived neurotrophic factor in human saliva. Arch Oral Bio. 2009 Jul 1;54(7):689-695.

[19] Scheurer PA, Firestone AR, Bürgin WB. Perception of pain as a result of orthodontic treatment with fixed appliances. Eur J Orthod. 1996 Jan 1;18(1):349-357.

[20] Krishnan V, Davidovitch Z. On a path to unfolding the biological mechanisms of orthodontic tooth movement. J Dent Res. 2009 Jul;88(7):597-608.

[21] Wahab RA, Kasim NA, Senafi S, Jemain AA, Abidin IZ, Shahidan MA, Ariffin SH. Enzyme activity profiles and ELISA analysis of biomarkers from human saliva and gingival crevicular fluid during orthodontic tooth movement using self-ligating brackets. Oral health Dent. Manage.2014;13: 194-199.

[22] Maltha JC, Van Leeuwen EJ, Dijkman GE, Kuijpers-Jagtman AM. Incidence and severity of root resorption in orthodontically moved premolars in dogs. Orthodontics & craniofacial research. 2004 May;7(2):115-121.

[23] Krishnan V, Davidovitch ZE. Cellular, molecular, and tissue-level reactions to orthodontic force. Am J Orthod Dentofacial Orthop. 2006 Apr 1;129(4):469-4e1.

[24] Perinetti G, Paolantonio M, D'Attilio M, D'Archivio D, Tripodi D, Femminella B, Festa F, Spoto G. Alkaline phosphatase activity in gingival crevicular fluid during human orthodontic tooth movement. Am J Orthod Dentofacial Orthop. 2002 Nov 1;122(5):548-556.

[25] Burstone CJ. The biomechanics of tooth movement. Vistas in orthodontics. 1962:197-213.

[26] Dolce C, Malone JS, Wheeler TT. Current concepts in the biology of orthodontic tooth movement. Semin Orthod2002 Mar 1 (Vol. 8, No. 1, pp. 6-12). WB Saunders.

[27] Pilon JJ, Kuijpers-Jagtman AM, Maltha JC. Magnitude of orthodontic forces and rate of bodily tooth movement. An experimental study. Am J Orthod Dentofacial Orthop. 1996 Jul 1;110(1):16-23.

[28] Van Leeuwen EJ, Maltha JC, Kuijpers-Jagtman AM. Tooth movement with light continuous and discontinuous forces in beagle dogs. Eur J Oral Sci. 1999 Dec;107(6):468-474.

[29] Zainal Ariffin SH, Yamamoto Z, Abidin Z, Megat Abdul Wahab R, Zainal Ariffin Z. Cellular and molecular changes in orthodontic tooth movement. Sci World J. 2011;11:1788-1803.

[30] Kaufman E, Lamster IB. The diagnostic applications of saliva—a review. Critical Reviews in oral biology & medicine. 2002 Mar;13(2):197-212.

[31] Navazesh M. Methods for collecting saliva. Annals of the New York Academy of Sciences. 1993 Sep;694(1):72-77.

[32] Streckfus CF, Storthz KA, Bigler L, Dubinsky WP. A comparison of the proteomic expression in pooled saliva specimens from individuals diagnosed with ductal carcinoma of the breast with and without lymph node involvement. Journal of oncology. 2009;

[33] Ellias MF, Zainal Ariffin SH, Karsani SA, Abdul Rahman M, Senafi S, Megat Abdul Wahab R. Proteomic analysis of saliva identifies potential biomarkers for orthodontic tooth movement. Sci World J. 2012;2012.

[34] Grieve III WG, Johnson GK, Moore RN, Reinhardt RA, DuBois LM. Prostaglandin E (PGE) and interleukin-1β (IL-1β) levels in gingival crevicular fluid during human orthodontic tooth movement. Am J Orthod Dentofacial Orthop. 1994 Apr 1;105(4):369-374.

[35] Uematsu S, Mogi M, Deguchi T. Interleukin (IL)-1β, IL-6, tumor necrosis factor-α, epidermal growth factor, and β2-microglobulin levels are elevated in gingival crevicular fluid during human orthodontic tooth movement. J Dent Res. 1996 Jan;75(1):562-567.

[36] Waddington RJ, Embery G. Proteoglycans and orthodontic tooth movement. J Orthod. 2001 Dec;28(4):281-290.

[37] Perinetti G, Varvara G, Salini L, Tetè S. Alkaline phosphatase activity in dental pulp of orthodontically treated teeth. Am J Orthod Dentofacial Orthop. 2005 Oct 1;128(4):492-496.

[38] Shibutani T, Murahashi Y, Tsukada E, Iwayama Y, Heersche JN. Experimentally induced periodontitis in beagle dogs causes rapid increases in osteoclastic resorption of alveolar bone. J Perio. 1997 Apr 1;68(4):385-391.

[39] Serra E, Perinetti G, D'Attilio M, Cordella C, Paolantonio M, Festa F, Spoto G. Lactate dehydrogenase activity in gingival crevicular fluid during orthodontic treatment. Am J Orthod Dentofacial Orthop. 2003 Aug 1; 124(2):206-211.

[40] Rohaya MA, Hisham ZS, Khazlina K. Preliminary study of aspartate aminotransferase activity in gingival crevicular fluids during orthodontic tooth movement. J Applied Sci. 2009;9(7):1393-1396.

[41] Ariffin SH, faiz ellias M, Wahab RM, Bakar Y, Senafi S. Profil aktiviti laktat dehidrogenase, asid fosfatase rintang tartarat dan alkalin fosfatase pada air liur semasa rawatan ortodontik. Sains Malaysiana. 2010 Jun;39(3):405-412.

[42] Zhang J, Zhou S, Zheng H, Zhou Y, Chen F, Lin J. Magnetic bead-based salivary peptidome profiling analysis during orthodontic treatment durations. Biochemical and biophysical research communications. 2012 May 18;421(4):844-849.

[43] Zelles T, Purushotham KR, Macauley SP, Oxford GE, Humphreys-Beher MG. Concise review: saliva and growth factors: the fountain of youth resides in us all. J Dent Res. 1995 Dec;74(12):1826-1832.

[44] Shugars DC, Wahl SM. The role of the oral environment in HIV-1 transmission. J Am Dent Asso. 1998 Jul 1;129(7):851-858.

[45] Mandel ID. The functions of saliva. J Dent Res. 1987 Feb;66(1_suppl):623-7.

[46] FDI Working Group 10, Core (1992). Saliva: its role in health and disease. Int Dent J 42:287-304.

[47] Fox PC. Saliva composition and its importance in dental health. Compendium (Newtown, Pa.). Supplement. 1989(13):S457.

[48] Mandel ID, Wotman S. The salivary secretions in health and disease. Oral sciences reviews. 1976(8):25-47.

[49] Sreebny LM (1989). Salivary flow in health and disease. Compend Suppl 13:461-469.

[50] Mandel ID. Salivary diagnosis: more than a lick and a promise. Journal of the American Dental Association (1939). 1993 Jan;124(1):85-7.

[51] Navazesh M. Methods for collecting saliva. Annals of the New York Academy of Sciences. 1993 Sep;694(1):72-77.

[52] Baum BJ. Principles of saliva secretion. Annals of the New York Academy of Sciences. 1993 Sep;694(1): 17-23.

[53] Young JA, Van Lennep EW. 1978. The process of secretion of organic products.

[54] Castle D. Cell biology of salivary protein secretion. In: Dobrosielski-Vergona K, editor. Biology of the salivary glands. Boca Raton, FL: CRC Press Inc.; 1993. pp. 81-104

[55] Turner RJ. Mechanisms of fluid secretion by salivary glands. Annals of the New York Academy of Sciences. 1993 Sep;694(1):24-35.

[56] Turner RJ, Paulais M, Manganel M, Lee III S, Moran A, Melvin JE. Ion and water transport mechanisms in salivary glands. Critical Reviews in Oral Biology & Medicine. 1993 Apr;4(3):385-391.

[57] Baum BJ. Principles of saliva secretion. Annals of the New York Academy of Sciences. 1993 Sep;694(1):17-23.

[58] Ambudkar IS. Regulation of calcium in salivary gland secretion. Critical Reviews in Oral Biology & Medicine. 2000 Jan;11(1):4-25.

[59] Bailey B, Klein J, Koren G. Noninvasive methods for drug measurement in pediatrics. Pediatric Clinics of North America. 1997 Feb 1;44(1):15-26.

[60] Olteanu C, Muresan A, Daicoviciu D, Tarmure V, Olteanu I, Keularts IM. Variations of some saliva markers of the oxidative stress in patients with orthodontic appliances. Fiziologia-Physiology. 2009 Sep 1;19(3).

[61] Sreebny LM, Schwartz SS. A reference guide to drugs and dry mouth–2nd edition. Gerodontology. 1997 Jul;14(1):33-47.

[62] Fox PC. Acquired salivary dysfunction: drugs and radiation. Annals of the New York Academy of Sciences. 1998 Apr;842(1):132-137.

[63] Chauncey HH (1961). Salivary enzymes. J Am Dent Assoc 63:361-369.

[64] Fleissig Y, Deutsch O, Reichenberg E, Redlich M, Zaks B, Palmon A, Aframian DJ. Different proteomic protein patterns in saliva of Sjögren's syndrome patients. Oral diseases. 2009 Jan;15(1):61-68.

[65] Vitorino R, Lobo MJ, Ferrer-Correira AJ, Dubin JR, Tomer KB, Domingues PM, Amado FM. Identification of human whole saliva protein components using proteomics. Proteomics. 2004 Apr;4(4):1109-1115.

[66] Zreiqat H, Howlett CR, Gronthos S, Hume D, Geczy CL. S100A8/S100A9 and their association with cartilage and bone. J Mol Histo. 2007 Oct 1;38(5):381-391.

[67] Van Lent PL, Grevers L, Blom AB, Sloetjes A, Mort JS, Vogl T, Nacken W, Van Den Berg WB, Roth J. Myeloid-related proteins S100A8/S100A9 regulate joint inflammation and cartilage destruction during antigen-induced arthritis. Annals of the rheumatic diseases. 2008 Dec 1;67(12):1750-1758.

[68] Udby L, Sørensen OE, Pass J, Johnsen AH, Behrendt N, Borregaard N, Kjeldsen L. Cysteine-rich secretory protein 3 is a ligand of α1B-glycoprotein in human plasma. Biochemistry. 2004 Oct 12;43(40):12877-12886.

[69] Kosari F, Asmann YW, Cheville JC, Vasmatzis G. Cysteine-rich secretory protein-3: a potential biomarker for prostate cancer. Cancer Epidemiology and Prevention Biomarkers. 2002 Nov 1;11(11):1419-1426.

[70] Gonçalves LD, Soares MR, Nogueira FC, Garcia C, Camisasca DR, Domont G, Feitosa AC, de Abreu Pereira D, Zingali RB, Alves G. Comparative proteomic analysis of whole saliva from chronic periodontitis patients. Journal of proteomics. 2010 May 7;73(7):1334-1341.

[71] Ianzer D, Konno K, Xavier CH, Stöcklin R, Santos RA, de Camargo AC, Pimenta DC. Hemorphin and hemorphin-like peptides isolated from dog pancreas and sheep brain are able to potentiate bradykinin activity in vivo. Peptides. 2006 Nov 1;27(11):2957-2966.

[72] Lu J, Stewart AJ, Sadler PJ, Pinheiro TJ, Blindauer CA. Albumin as a zinc carrier: properties of its high-affinity zinc-binding site. 2008: 1317-1321.

[73] Suárez-Bonnet A, de las Mulas JM, Herráez P, Rodríguez F, de los Monteros AE. Immunohistochemical localisation of 14-3-3 σ protein in normal canine tissues. The Veterinary Journal. 2010 Aug 1;185(2):218-221.

[74] Buczko P, Zalewska A, Szarmach I. Saliva and oxidative stress in oral cavity and in some systemic disorders. J Physiol Pharmacol. 2015 Feb;66:1-7.

[75] Atuğ Özcan SS, Ceylan I, Özcan E, Kurt N, Dağsuyu İM, Canakci CF. Evaluation of oxidative stress biomarkers in patients with fixed orthodontic appliances. Disease markers. 2014;2014.

[76] Szarmach IJ, Buczko P, Tankiewicz-Kwedlo A, Kasacka I, Tankiewicz J, Pawlak D. The concentration of kynurenine and anthranilic acid in saliva of patients with fixed orthodontic appliances. Pol J Environ Stud. 2007;16:125-127.

[77] Roberts WE, Huja S, Roberts JA. Bone modeling: biomechanics, molecular mechanisms, and clinical perspectives. Semin Orthod2004 Jun 1 (Vol. 10, No. 2, pp. 123-161). WB Saunders.

[78] Yamashiro T, Fukunaga T, Kabuto H, Ogawa N, Takano-Yamamoto T. Activation of the bulbospinal serotonergic system during experimental tooth movement in the rat. J Dent Res. 2001 Sep;80(9):1854-1857.

[79] Hattori Y, Watanabe M, Iwabe T, Tanaka E, Nishi M, Aoyama J, Satoda T, Uchida T, Tanne K. Administration of MK-801 decreases c-Fos expression in the trigeminal sensory nuclear complex but increases it in the midbrain during experimental movement of rat molars. Brain research. 2004 Sep 24;1021(2): 183-191.

[80] Gaur A, Maheshwari S, Verma SK. Detection of molecular biomarkers as a

diagnostic tool in the planning and progression of orthodontic treatment. J Orofacial Sci. 2017 Jul 1;9(2):80.

[81] Ferguson DB. Oral bioscience. Churchill livingstone: China; 1999

[82] Sugiyama Y, Yamaguchi M, Kanekawa M, Yoshii M, Nozoe T, Nogimura A, Kasai K. The level of cathepsin B in gingival crevicular fluid during human orthodontic tooth movement. Eur J Orthod. 2003 Feb 1;25(1):71-76.

[83] Waddington RJ, Embery G. Proteoglycans and orthodontic tooth movement. J Orthod. 2001 Dec;28(4): 281-290.

[84] Last KS, Stanbury JB, Embery G. Glycosaminoglycans in human gingival crevicular fluid as indicators of active periodontal disease. Arch Oral Bio. 1985 Jan 1;30(3):275-281.

[85] Uematsu S, Mogi M, Deguchi T. Interleukin (IL)-1β, IL-6, tumor necrosis factor-α, epidermal growth factor, and β2-microglobulin levels are elevated in gingival crevicular fluid during human orthodontic tooth movement. J Dent Res. 1996 Jan;75(1):562-567.

[86] Uematsu S, Mogi M, Deguchi T. Increase of transforming growth factor-β1 in gingival crevicular fluid during human orthodontic tooth movement. Arch Oral Bio. 1996 Nov 1;41(11):1091-1095.

[87] Lee KJ, Park YC, Yu HS, Choi SH, Yoo YJ. Effects of continuous and interrupted orthodontic force on interleukin-1β and prostaglandin E2 production in gingival crevicular fluid. Am J Orthod Dentofacial Orthop. 2004 Feb 1;125(2):168-177.

[88] Grieve WG III, Johnson GK, Moore RN, Reinhardt RA, Dubois LM. Prostaglandin E (PGE) and interleukin-1 beta (IL-1beta) levels in gingival crevicular fluid during human orthodontic tooth movement. Am J Orthod Dentofacial Orthop 1994;105: 369-374.

[89] Lowney JJ, Norton LA, Shafer DM, Rossomando EF. Orthodontic forces increase tumor necrosis factor α in the human gingival sulcus. Am J Orthod Dentofacial Orthop. 1995 Nov 1;108(5):519-524.

[90] Griffiths GS, Moulson AM, Petrie A, James IT. Evaluation of osteocalcin and pyridinium crosslinks of bone collagen as markers of bone turnover in gingival crevicular fluid during different stages of orthodontic treatment. J clinical perio. 1998 Jun;25(6):492-498.

[91] Insoft M, King GJ, Keeling SD. The measurement of acid and alkaline phosphatase in gingival crevicular fluid during orthodontic tooth movement. Am J Orthod Dentofacial Orthop. 1996 Mar 1;109(3):287-296.

[92] Perinetti G, Paolantonio M, D'Attilio M, D'Archivio D, Dolci M, Femminella B, Festa F, Spoto G. Aspartate aminotransferase activity in gingival crevicular fluid during orthodontic treatment. A controlled short-term longitudinal study. J Perio. 2003 Feb 1;74(2):145-152.

[93] Serra E, Perinetti G, D'Attilio M, Cordella C, Paolantonio M, Festa F, Spoto G. Lactate dehydrogenase activity in gingival crevicular fluid during orthodontic treatment. Am J Orthod Dentofacial Orthop. 2003 Aug 1;124(2):206-211.

[94] Apajalahti S, Sorsa T, Railavo S, Ingman T. The in vivo levels of matrix metalloproteinase-1 and-8 in gingival crevicular fluid during initial

orthodontic tooth movement. J Dent Res. 2003 Dec;82(12):1018-1022.

[95] Allgayer S, Macedo de Menezes L, Rinaldi L, Roennau M. Cytokines in crevicular fluid and orthodontic force: a systematic review. Revista Odonto Ciência. 2017 Jun 1;32(2).

[96] Redlich M, Rahamim E, Gaft A, Shoshan S. The response of supraalveolar gingival collagen to orthodontic rotation movement in dogs. Am J Orthod Dentofacial Orthop. 1996 Sep 1; 110(3):247-255.

[97] Alhashimi N, Frithiof L, Brudvik P, Bakhiet M. Orthodontic tooth movement and de novo synthesis of proinflammatory cytokines. Am J Orthod Dentofacial Orthop. 2001 Mar 1;119(3):307-312.

[98] Redlich M, Reichenberg E, Harari D, Zaks B, Shoshan S, Palmon A. The effect of mechanical force on mRNA levels of collagenase, collagen type I, and tissue inhibitors of metalloproteinases in gingivae of dogs. J Dent Res. 2001 Dec;80(12):2080-2084.

[99] Capelli Junior J, Kantarci A, Haffajee A, Teles RP, Fidel Jr R, Figueredo CM. Matrix metalloproteinases and chemokines in the gingival crevicular fluid during orthodontic tooth movement. Eur J Orthod. 2011 Mar 9;33(6):705-711.