EVALUATION OF GINGIVAL CREVICULAR FLUID AND SALIVA AS COMPARATIVE MARKERS IN THE EXPRESSION OF INTERLEUKIN - 1 β DURING ORTHODONTIC TOOTH MOVEMENT - AN IN-VIVO STUDY

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CERTIFICATE

This is to certify that this dissertation titled **"EVALUATION OF GINGIVAL CREVICULAR FLUID AND SALIVA AS COMPARATIVE MARKERS IN THE EXPRESSION OF INTERLEUKIN -1β DURING ORTHODONTIC TOOTH MOVEMENT – AN IN-VIVO STUDY"** is a bonafide record of work done by **Dr. SIVASUBRAMANIAN.J** under my guidance during his postgraduate study period 2010–2013.

This dissertation is submitted to **THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY**, in partial fulfillment for the degree of **Master of Dental Surgery** in Branch V – Orthodontics and Dentofacial Orthopaedics. It has not been submitted (partially or fully) for the award of any other degree or diploma.

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EVALUATION OF GINGIVAL CREVICULAR FLUID AND SALIVA AS COMPARATIVE MARKERS IN THE EXPRESSION OF INTERLEUKIN -1β DURING ORTHODONTIC TOOTH MOVEMENT – AN IN-VIVO STUDY

ABSTRACT

OBJECTIVE: This study was undertaken to correlate the findings of presence of Interleukin - 1β in GCF and saliva during initial levelling stage of orthodontic treatment and to substantiate if saliva as a medium could be used as a suitable marker for establishing the expression of IL - 1β.

MATERIALS AND METHODS: GCF and Whole Saliva samples were collected from ten orthodontic patients (6 females and 4 males, aged 15 - 25 yrs) who had Little's Irregularity Index score (≥ 10) referring to very severe irregularity and had undergone upper and lower first bicuspid extractions. The samples were collected at the following stages:- T 0 – Pre Treatment, T 1 – 7 days after initiation of orthodontic treatment, T 2 - 30 days after initiation of orthodontic treatment. Disposable micropipettes were used for GCF collection. The saliva samples were collected in sterile containers. The collected samples were subjected to an ELISA test to determine the concentrations of IL - 1 β.

RESULTS: The results of our study showed that IL - 1β levels were highly increased on the 7^{th} day in both GCF and Saliva (GCF - 288.57 +/- 111.88 Pg/ml, Saliva - 272.50 +/- 108.16 Pg/ml) after appliance activation when compared to the baseline levels

(GCF - 151.20 +/- 129.21 Pg/ml, Saliva - 186.30 +/- 163.57 Pg/ml), , and they gradually decreased on the 30th day (GCF - 228.67 +/- 103.37 Pg/ml, Saliva - 211.75 +/- 120.09 Pg/ml) when compared to the realtively increased levels seen on 7th day. No statistically significant results could be drawn because of high amount of inter individual variability.

CONCLUSION: Results of the current study, show a positive indication that saliva could be used as a viable substitute for GCF when used for assessing bone remodelling for patients undergoing orthodontic treatment.

KEY WORDS: Orthodontic tooth movement; Little's Irregularity Index; Gingival crevicular fluid; Whole Saliva; Interleukin-1β

Introduction

INTRODUCTION

The cascade of biological events that induce orthodontic tooth movement is initiated by mechanical stresses in the periodontium. Forces are transferred to the teeth by the clinician using appliances designed to displace teeth by a prescribed amount and in a desired direction. This force sends signals to the cells to remodel tissues in a way that allows teeth to move. To interpret the biological responses to activation of any orthodontic appliance, each interface in the process must be thoroughly understood.[52]

The classic pioneering studies of such luminaries as ***Oppenheim***[68,69]***, Reitan***[74,75]***, Rygh***[82]***, and Davidovitch et al***[15,16] still form the basis of the orthodontist's knowledge in this area. Their studies reported on details that were noted but have not been sufficiently emphasized. These observations have implications in the understanding of events seen in more recent cell biology studies.

One of the most significant advances in connective tissue biology during the 1980s was the demonstration that many **cytokines**, in addition to mediating the host immunological response to exogenous antigens, were also produced by connective tissue cells such as fibroblasts and osteoblasts and involved in normal physiological turnover and bone remodelling. Cytokines are low-molecular weight proteins (mw < 25 kDa) produced by cells that regulate or modify the action of other cells in an autocrine (acting on the cell of origin) or paracrine (acting on adjacent cells) manner. The definition includes the Interleukins (ILs), Tumour necrosis factors (TNFs), Interferons, Growth factors, and Colony stimulating factors. The first cytokine shown to play a role in bone turnover was IL-1, which in addition to activating lymphocytes

also proved to be a potent bone resorptive agent. Parathyroid hormone and other systemic hormones as well as cytokines, such as IL-1, TNF- α , and IL-6, stimulate bone resorption by their ability to upregulate RANKL expression by osteoblasts/stromal cells[60]. ***Masella RS and Meister M[59]*** stated that the earliest marker of bone resorption could be the cytokine interleukin-1beta (IL-1β).

The **most potent** among these cytokines is **IL-1**, which directly stimulates osteoclast function through IL-1 type 1 receptor, expressed by osteoclasts[45]. Secretion of IL-1 is triggered by various stimuli, including neurotransmitters, bacterial products, other cytokines, and mechanical forces[16]. IL-1 has 2 forms—α and β—that code different genes. These interleukins have been reported to have similar biologic actions, systemically and locally.[17,18] These actions include attracting leukocytes and stimulating fibroblasts, endothelial cells, osteoclasts, and osteoblasts to promote bone resorption and inhibit bone formation[83]. Osteoblasts are target cells for IL-1, which in turn conveys messages to osteoclasts to resorb bone.[79]

After numerous In-Vitro studies[84,85,86,26,72] and animal experiments on the effect Il - 1β on bone remodeling[87], the first In-Vivo study was that of ***Grieve et al[29]*** who reported that the IL-1β and Prostaglandin E (PGE) levels in human GCF were elevated during the first 24 hours of tooth movement after force application and returned to baseline in 7 days. ***Uematsu et al[107]*** showed similar results regarding ILs, tumor necrosis factor α, and epidermal growth factor. They assumed that the restored cytokine levels might be due to a lack of force consistency.

Over the past few years In-Vivo studies in the Biology of Orthodontic Tooth Movement have used **Gingival Crevicular Fluid (GCF)** because of its noninvasive nature and ease of repetitive sampling from the same site with the help of platinum loops, filter paper strips, gingival washings, and **micro pipettes**[42,45,66,109]. The fluid can be used to analyze various biochemical markers such as prostaglandin production and the action of various extracellular and intracellular factors, such as IL-1, IL-6, TNF-α, epidermal growth factors, β2 microglobulin, cathepsin, aspartate aminotransferease, alkaline phosphatase, and lactate dehydrogenase.[6,20,21,45 ,70,92,101,113]

Although gingival crevicular fluid might be a potential candidate for assessing inflammatory markers, there are certain limiting factors which include different sampling sites in oral cavity and the differences in sites must be considered. In addition the sampling technique is not easy and a long time is needed for collection of sample.[67]

Over the past 10 years, the use of **saliva as a diagnostic fluid** has gained attention in the medical and dental field and has become a translational research success story. Saliva contains many enzymes and some inflammatory markers and no specific laboratory devices are needed for collection of saliva. There is minimal risk of contracting infections during saliva collection, and saliva can be used in clinically challenging situations, such as obtaining samples from children or handicapped or anxious patients.[71]

Clinical studies have confirmed that Interleukin - 1β can be detected in human gingival crevicular fluid (GCF) during orthodontics but there is **lack of literature**

whether the same IL - 1β can be detected in saliva during orthodontic tooth movement.

Most of the studies regarding the assessment of GCF for IL - 1β during orthodontic tooth movement have applied heavy distalization forces on tooth. As a result of the great force that is applied to the tooth, a hyalinized zone occurs in the compressed periodontal ligament. This hyaline zone has also been described as an area of focal aseptic necrosis[27]. From the tension side, an indirect resorption process initiates and induces IL- 1β, which regulates bone resorption. But orthodontic treatment does not always use distalization forces that produce hyalinization as is considered in these studies.

However, in the first stages of orthodontic treatment, low forces are used that do not result in hyalinized zones and indirect resorption[7]. Therefore, there may be different levels of cytokines in the leveling and retraction stages of the orthodontic treatment.

Literature is scant when it comes to assessment of IL - 1β in GCF during leveling stages of orthodontic tooth movement and there are only few such studies[7,44].

Keeping this in mind the **aim of the study** was**:**

a) To correlate the findings of presence of Interleukin - 1β in GCF and saliva during initial leveling stage of orthodontic treatment and

b) To substantiate if saliva as a medium could be used as a suitable marker for establishing the expression of Interleukin - 1β.

Review of Literature

REVIEW OF LITERATURE

LITTLE IRREGULARITY INDEX

Little (1975)[53]- It is done by measuring the linear displacement of the anatomic contact points (as distinguished from the clinical contact points) of each mandibular incisor from the adjacent tooth anatomic point on the study model with the help of a dial caliper calibrated to at least tenths of a millimeter. This gives in total five reading and the sum of these five displacements represents the relative degree of anterior irregularity. Care should be taken that the caliper is held parallel to the occlusal plane while the beaks are lined up with the contact points to be measured to ensure that the displacement only in the horizontal direction is recorded. After five measurements are obtained total of all measurements is done. Perfect alignment from the mesial aspect of left canine to mesial aspect of the right canine would have a score of 0, with the increased crowding represented by greater displacement and, therefore a higher score. The severity of crowding is graded into five categories as follow:0 - No crowding, 1 - 3 – Mild crowding, 4 - 6 - Moderate crowding, 7 – 9 - Severe crowding, 10 – Very severe crowding.

TISSUE REACTION TO ORTHODONTIC TOOTH MOVEMENT

Harris (1839)[31] stated that if, there be any pressure against a tooth, it causes an absorption of the side of the alveolus against which its fang is pressed. But this does not necessarily destroy the socket, for as the internal paries is carried off by the absorbents,

the external of the same side is thickened by a deposition of new bone; and the vacuum thus made on the opposite side, is also filled up.

Sandstedt C (1904 – 1905)[88,89] in his experimental model used a labial arch was bent to engage the six maxillary incisors of a dog and inserted into horizontal tubes attached to bands on the canines. The appliance was activated over a 3-week period by screws distal to the buccal tubes and during that time the crowns of the incisors were moved lingually by 3 mm. Sandstedt found that bone was deposited on the alveolar wall on the tension side of the tooth with both heavy and light forces, and that the newly formed bone spicules followed the orientation of the periodontal fibre bundles. On the pressure side, with light forces, alveolar bone was resorbed directly by numerous multinucleate osteoclasts in Howship's lacunae. With heavy forces, the periodontal tissues were compressed, leading to capillary thrombosis, cell death, and the production of localized cell-free areas of what he called hyalinization (owing to its glasslike appearance resembling hyaline cartilage in histological sections). At these sites, osteoclastic resorption of the adjacent alveolar wall did not take place directly, but was initiated by a process referred to by Sandstedt as ' undermining resorption ' from the neighbouring marrow spaces.

Oppenheim (1911, 1930)[68,69] published the results of experimental work carried out on the primary teeth of monkeys. He found that where a tooth had been tipped labially, the original bone disappeared completely from the labial surface and was replaced by new bone. He concluded that bone tissue, be it compact or cancellated, reacts to pressure by a transformation of its entire architecture; this takes place by resorption of the bone present and deposition of new bone tissue; both processes occur simultaneously. Deposition finally preponderates over resorption.

Schwarz (1932)[93] attempted to explain the difference between the findings of Sandstedt and Oppenheim by the fact that Oppenheim had killed his animals several days after the appliance had been last activated and did not therefore see the acute effect of the applied force, only a stage of regeneration after the force had been exhausted. It seems more likely that what Oppenheim was describing was the response of the periosteal and endosteal bone surfaces to the bending of the labial alveolar plate. The labial alveolar bone is particularly thin in his illustrations and would have been easily deformed in a monkey in the primary dentition.

Reitan (1957, 1964)[74,75] made extensive use of human material, particularly premolars that were destined for orthodontic extractions. His work highlighted the complexity of the tissue response to orthodontic treatment depending upon (1) the type (continuous versus intermittent) and magnitude of the force applied, (2) the mechanics involved (tipping versus bodily movement), and (3) the variation in tissue reaction between individual patients. He observed that during the initial stages of a tipping movement, cell free or hyalinized areas were frequently created with a continuous force of 30 g. The time taken to remove such tissue by undermining resorption varied from 2 to 4 weeks and occasionally longer depending on the length of the root. Intermittent forces of 70 – 100 g were also found to produce hyalinization. Cell-free areas were more common in tipping than in bodily movements presumably because, in the latter, the force was more evenly distributed along the root – bone interface. These experiments showed that even with an applied force as low as 30 g, some degree of hyalinization and root resorption appeared inevitable.

OPTIMAL ORTHODONTIC FORCE

Schwarz (1932)[93] gave the classic definition of optimal force as "the force leading to a change in tissue pressure that approximated the capillary vessels' blood pressure, thus preventing their occlusion in the compressed periodontal ligament." According to Schwarz, forces below optimum produce no reaction, whereas forces above that level lead to tissue necrosis, thus preventing frontal resorption of the alveolar bone.

Storey and Smith (1952)[100] in an investigation involving nine patients, found that movement of the canine teeth into premolar extraction sites occurred rapidly when the value of the applied force was in the range of 150 – 250 g (5 – 9 ounces); however, below 150 g, the canines did not move significantly. When the springs were activated to apply forces in the range of 400 – 600 g (14 – 21 ounces), the anchor teeth (molars and second premolars) moved forward, with the canines remaining relatively stationary.

Daskalogiannakis J (2000)[14] defined Orthodontic force as "force applied to teeth for the purpose of effecting tooth movement, generally having a magnitude lower than an orthopedic force," whereas orthopedic force is defined as "force of higher magnitude in relation to an orthodontic force, when delivered via teeth for 12 to 16 hours a day, is supposed to produce a skeletal effect on the maxillofacial complex."

THE ROLE OF INTERLEUKIN 1β IN BONE REMODELLING

Dinarello CA (1988)[17] stated that IL-1 is a polypeptide product of various cells that mediates several components of the acute-phase response to infection and injury. Its most dramatic biological property is its ability to induce arachidonate metabolites in a variety of cells including PGE in the brain, fibroblasts, synovial cells, and chrondrocytes; in addition, IL-1 induces lipoxygenase products in lymphocytes and other cells. IL-1 has been cloned. There are two forms. The predominant form of IL-1 from human monocytes has a pI of 7 (also called beta) and is initially synthesized as a precursor molecule (31 kD). A minor form (less than 100-fold) also exists (pI 5, also called alpha). IL-1 seems unique among the lymphokines and monokines in that there is no signal peptide sequence for cleavage. Depending on the stimulus, intracellular levels of precursor IL-1 can be high, whereas some cell activators result in large amounts of processed IL-1. Precursor IL-1 is cleaved into a 17.5-kD peptide, which is the predominant extracellular form. IL-1 induces prostaglandins and lymphocyte activation as well as many different biological activities. These include fever, PGE production, protease release from synovial cells and chondrocytes, bone resorption, acute-phase protein synthesis, and other effects.

Saito S, Saito M, Ngan P, Lanese R, Shanfeld J, Davidovitch Z (1990)[85] demonstrated that cultured human periodontal ligament fibroblasts showed synergistic elevations in the synthesis of prostaglandin E and production of cAMP by the administration of parathyroid hormone and cytokines (interleukin 1 alpha, -1 beta, or tumour necrosis factor-alpha). Unstimulated conditioned media derived from these fibroblasts contained bone-resorbing activity. In addition, conditioned media generated

by cytokine-or parathyroid hormone-treated fibroblasts showed further increases in bone-resorbing activity.

Saito S, Ngan P, Saito M, Lanese R, Shanfeld J, Davidovitch Z (1990)[86] determined the effects of the cytokines interleukin 1 beta (IL-1 beta), interleukin 1 alpha (IL-1 alpha), tumor necrosis factor alpha (TNF-alpha), and interferon gamma (IFN-gamma), alone or in paired combinations, on PGE production by near-confluent human periodontal ligament (PDL) fibroblasts in vitro. They stated that the interactions between these cytokines varied in degree, depending on the particular combinations of cytokines. In addition, the administration of cytokine combinations was found to be additive, synergistic, subtractive, or suppressive on the production of PGE by PDL fibroblasts, depending on the duration of incubation. These experiments demonstrate the importance of the consideration of the interplay between cytokines produced by mononuclear cells on the mechanisms that regulate the functions of PDL fibroblasts.

Saito S, Ngan P, Rosol T, Saito M, Shimizu H, Shinjo N, Shanfeld J, Davidovitch Z (1991)[87] determined the effect of intermittent pressure on bone resorption of human periodontal ligament (PDL) fibroblasts, cultured from extracted healthy premolars, and a cloned osteogenic cell line (MC3T3-E1). Cells (1 x 10(5] were incubated with BGJb medium in the presence or absence of the following factors: intermittent negative (-30 g/cm2) or positive (30 g/cm2) hydrostatic pressure and interleukin-1 beta (IL-1 beta, 1 ng/mL), for 24 h. Conditioned media (CM) generated from cultures of either cell types were used for prostaglandin E (PGE) assay, bone resorption assay, and assessment of osteoclast (OC)-like cell formation. Unstimulated PDL fibroblasts or MC3T3-E1 cells produced measurable amounts of PGE and bone-resorbing activity as measured by 45Ca released from mouse calvaria and

OC-like cells. IL-1 beta-treated cells showed significantly elevated levels of PGE, bone resorption, and OC-like cell formation, as compared with unstimulated cells. Intermittent positive pressure (IPP) alone stimulated PGE production, but the resultant CM did not stimulate bone resorption or OC-like cell formation when IPP was applied to either cell type. The application of IPP, together with IL-1 beta in CM, caused a slight increase in the number of alpha-like cells, as compared with that of IL-1 beta-treated CM in both cell types. On the other hand, direct application of IPP on mouse bone-marrow cultures significantly increased the number of OC-like cells. This effect was additive in combination with either CM from unstimulated cells or exogenous addition of PGE2.

Saito M, Saito S, Ngan PW, Shanfeld J, and Davidovitch Z (1991)[84] stated that cytokines are local mediators released by cells of the immune system in response to stimulation by a variety of agents. These polypeptides may interact directly or indirectly with bone ceils. The objectives of their study were (1) to localize prostaglandin E (PGE) and the cytokine interleukin-1β (IL-1β) in the periodontal ligament after the application of mechanical force to teeth in rive and (2) to determine the effects of mechanical stress or I1-1β (or the two in combination) on PGE synthesis and bone resorption by fibroblasts in the human periodontal ligament (PDL). In 24 female cats, one maxillary canine was tipped distally by 80 gm force for 12 hours, 24 hours, or 7 days. PGE and 11-1β were localized immunohistochemically in serial jaw sections, and semiquantitation of cellular-staining intensity was done by microphotometry. Unstressed periodontal ligament cells stained mildly for PGE and IL-1β, but the staining intensity increased significantly in sites of tension. Human periodontal ligament fibroblasts were preincubated with mechanical stress and/or

IL-1β in the presence or absence of indomethacin for 1 hour. Then the media were replaced by BGJ~ (Fitton-Jackson modification) medium (GIBCO), and incubation was continued for 4, 8, or 24 hours in conditioned media. PGE concentrations in conditioned media were determined by radioimmunoassay, and bone-resorbing activity in conditioned media was assessed by ~Ca release from prelabeled neonatal mouse calvaria. The conditioned media derived from cells stimulated by mechanical stress plus IL-1β caused significantly more bone resorption than the conditioned media obtained from cells that had been treated by each factor alone. The addition of indomethacin did not inhibit bone resorption completely. These results demonstrate that periodontal ligament cells respond to mechanical stress by increased production of PGE, and that I1 - 1β enhances this response.

Davidovitch Z (1991)[15] described that with respect to bone metabolism, cytokines with demonstrated or suspected effects are IL-1, IL-2, IL-3, IL-6, TNF-α, and IFN-γ. Of these cytokines, the most potent stimulator of bone resorption in vitro is IL-1. It is produced by many cell types, including osteoblasts and chondrocytes. Secretion of IL-1 is triggered by a variety of stimuli, including other cytokines and whole microorganisms. It has two distinct forms, IL-lα and IL-1β.

Uitto VJ and Larjava H (1991)[108] stated that Type I collagen is the major structural component of many connective tissues, including gingiva, periodontal ligament, dentin, cementum, and bone. Type Ill and V collagens may have relevance to inflammation and tissue regeneration. Certain cytokines, such as transforming growth factor 1B and interleukin- 1, released from inflammatory cells may selectively regulate the synthesis of different collagen types.

Sandy JR, Farndale RW, Meikle MC (1993)[91] stated that one of the major unexplained responses to orthodontic tooth movement is the resorption of bone in areas traditionally described as being under "pressure" and its deposition in areas of "tension." This raises the question; how do cells distinguish between tension and compression? When compressive or tensile forces were applied to rabbit tibiae, both resulted in new bone formation. If bone cells cannot distinguish between a tensile and a compressive mechanical stress, how is bone resorption mediated on the "compression" side of the alveolar process during orthodontic tooth movement? At The Biology of Tooth Movement conference held in Farmington, Conn., Nov. 5 to 7, 1986, we suggested that the answer was likely to be found in the field of cytokine biology: ~ According to this speculative hypothesis, whether the result is the formation or resorption of bone depends on (1) the cytokines produced locally by mechanically activated cells; and (2) the functional state of the available target cells. Cytokines that can influence connective tissue remodeling include the interleukins, tumor necrosis factors, interferons, polypeptide growth factors, and colony stimulating factors; the list continues to grow at an alarming rate. The recent immunolocalization of interleukin-lα (IL-lα) and interleukin-lβ (IL-1β) in the periodontaltissues of cat canine teeth after the application of a tipping force, has provided the first experimental evidence to support this hypothesis.

H. Birkedal-Hansen, W.G.I. Moore, M.K. Bodden, L.J. Windsor, B. Birkedal-Hansen, A. DeCarlo and J.A. Engler (1993)[35] in their review article stated that Interleukin - 1β induces the stimulation of Matrix Metalloproteinases (MMP) expression.

Dinarello CA (1994)[15] explained the biological properties of IL – 1. The polypeptide cytokine interleukin-1 (IL-1) affects nearly every tissue and organ system.

IL-1 is the prototype of the pro-inflammatory cytokines in that it induces the expression of a variety of genes and the synthesis of several proteins that, in turn, induce acute and chronic inflammatory changes. IL-1 is the term for two polypeptides (IL-lα and IL-1β) that possess a wide spectrum of inflammatory, metabolic, physiologic, hematopoietic, and immunologic properties.

Gemmell E and Seymour GJ (1994)[22] explained that Cytokines were first named on the basis of their activity in biological assays. The term "interleukin" was introduced to apply to factors with multiple overlapping biological activities.

C. Feliciani, A.K. Gupta and D.N. Saucier (1996)[13] described that IL-1 is synthesized by a wide variety of cells, including keratinocytes, fibroblasts, Langerhans cells, mesangial cells, B-lymphocytes, natural killer cells, endothelial cells, smooth-muscle cells, astrocytes, microglial cells, corneal, gingival, and thymic epithelial cells, and some T-cell lines.

Havemose-Poulsen A and Holmstrup P (1997)[33] in their review of literature stated that Monocytes/macrophages may be stimulated by putative periodontopathic bacteria or their products to synthesize interleukin-1 (IL-1). IL- 1 may potentiate its own expression, and the biological effect of IL-1 can be inhibited by interleukin- 1 receptor antagonists (IL- Ira). Systemic or locally produced IL-1 may mediate a shift in the cellular properties of host cells, such as the fibroblast. The cellular fibroblastic properties that may be altered in response to cytokine stimulation include: cell proliferation, phagocytosis, collagen synthesis, and the synthesis of proteolytic enzymes such as matrix metalloproteinases (MMPs) and their corresponding tissue inhibitors of metalloproteinases (TIMPs). This results in collagen metabolism and therby periodontal disease progression

Ghafari JG (1997)[23] stated that a burgeoning trend is the integration of basic biologic research and clinical research, clearly illustrated by the attempt to gauge therapeutic progress through biologic means. This premise is well documented in the analysis of crevicular fluids to detect biologic factors, such as two potent bone resorbing mediators, interleukin (IL) 1β and prostaglandin E 2, to evaluate the progress of tooth movement in the individual patient.

H. Okada and S. Murakami (1998)[36] explained the mechanisms by which cytokines act on the target cells. (a) Autocrine: a mechanism involving the synthesis of a cytokine by one cell that influences the growth and functional activities of the cells producing the cytokine via a specific receptor on its surface. (b) Intracrine: a mechanism involving the direct action of a cytokine within a cell. (c) Juxtacrine: a mechanism involving specific cell-to-cell contacts. It occurs via an interaction of a membrane-bound form of a cytokine that is normally secreted with a receptor on an adjacent cell. Another mode of juxtacrine interaction involves extracellular matrix-associated rather than membrane-anchored forms of cytokines. (d) Paracrine: a mechanism involving the synthesis of a cytokine by one cell that influences the rowth and functional activities of nearby cells expressing the receptor for this cytokine. Therefore, this mechanism differs from that mediated by endocrine factors, which are transported to the site of action by the circulation. IL-1 is known to stimulate the proliferation of keratinocytes, fibroblasts, and endothelial cells and to enhance fibroblast synthesis of type I procollagen, collagenase, hyaluronate, fibronectin, and prostaglandin E2 IL-I is, therefore, a critical component in the homeostasis of periodontal tissues. However, unrestricted production of IL-1 may lead to tissue damage by enhancement of bone resorption, stimulation of metalloproteinase

production, stimulation of plasminogen activator and stimulation of prostaglandin synthesis

Kyrkanides S, O'Banion MK, and Subtelny JD (2000)[47] stated that the use of over-the-counter nonsteroidal anti-inflammatory drugs during tooth movement may result in aberrant remodeling of periodontal vasculature and other structures, ultimately affecting orthodontic treatment efficacy. Cyclooxygenase inhibition resulted in exacerbation of IL-1β–mediated collagenase B (MMP-9) production and activity, as well as attenuation of type IV procollagen synthesis levels by endothelial cells in vitro.

Alhashimi N, Frithiof L, Brudvik P and Bakhiet M (2001)[2] performed in situ hybridization was performed to measure the messenger RNA expression of IL-1β, IL-6, and TNF- α at 3, 7, and 10 days after the application of orthodontic force on the maxillary first molars of 12 rats. The contralateral side and 3 untreated rats served as controls. Measurements of the messenger RNA expression were selected as the means to investigate the role of orthodontic force in de novo synthesis of proinflammatory cytokines. After the application of force, the induction of IL-1β and IL-6 was observed to reach a maximum on day 3 and to decline thereafter. No messenger RNA induction of either cytokine was measured in the control teeth. The messenger RNA expression of TNF-α was not detected at any time point of this study in the experimental or contralateral sides or in the control animals. Their data supported the hypothesis that these proinflammatory cytokines may play important roles in bone resorption after the application of orthodontic force.

Masella RS and Meister M (2006)[59] stated that the earliest marker of bone resorption could be the cytokine interleukin-1beta (IL-1β). A mutant gene of IL-1β might be associated with down-regulation of this important cytokine. Osteoclastic bone

resorption is also facilitated by PGE2, nitric oxide, IL-6, and other inflammatory cytokines.

Meikle MC (2006)[60] stated that one of the most significant advances in connective tissue biology during the 1980s was the demonstration that many cytokines, in addition to mediating the host immunological response to exogenous antigens, were also produced by connective tissue cells such as fibroblasts and osteoblasts and involved in normal physiological turnover and bone remodelling. Cytokines are low-molecular weight proteins (mw < 25 kDa) produced by cells that regulate or modify the action of other cells in an autocrine (acting on the cell of origin) or paracrine (acting on adjacent cells) manner. The definition includes the interleukins (ILs), tumour necrosis factors (TNFs), interferons, growth factors, and colonystimulating factors. The first cytokine shown to play a role in bone turnover was IL-1, which in addition to activating lymphocytes also proved to be a potent bone resorptive agent. PTH and other systemic hormones as well as cytokines, such as IL-1, TNF- α , and IL-6, stimulate bone resorption by their ability to upregulate RANKL expression by osteoblasts/stromal cells.

Krishnan V and Davidovitch Z (2006)[45] stated that the early phase of orthodontic tooth movement always involves an acute inflammatory response, characterized by periodontal vasodilatation and migration of leucocytes out of the capillaries. These migratory cells produce various cytokines, the local biochemical signal molecules, that interact directly or indirectly with the entire population of native paradental cells. Cytokines, acting as paracrines or autocrines, always with other systemic and local signal molecules, evoke the synthesis and secretion of numerous substances by their target cells, including prostaglandins, growth factors, and

cytokines. Ultimately, these cells comprise the functional units that remodel the paradental tissues and facilitate tooth movement.

Cytokines are extracellular signaling proteins that act on nearby target cells in low concentrations in an autocrine or paracrine fashion in cell-to-cell communications. Cytokines that were found to affect bone metabolism, and thereby orthodontic tooth movement, include interleukin 1 (IL-1), interleukin 2 (IL-2) interleukin 3 (IL-3), interleukin 6 (IL-6), interleukin 8 (IL-8), tumor necrosis factor alpha (TNFα), gamma interferon (IFNγ) and osteoclast differentiation factor (ODF). The most potent among these is IL-1, which directly stimulates osteoclast function through IL-1 type 1 receptor, expressed by osteoclasts. Secretion of IL-1 is triggered by various stimuli, including neurotransmitters, bacterial products, other cytokines, and mechanical forces. IL-1 has 2 forms—α and β—that code different genes. These interleukins have been reported to have similar biologic actions, systemically and locally. These actions include attracting leukocytes and stimulating fibroblasts, endothelial cells, osteoclasts, and osteoblasts to promote bone resorption and inhibit bone formation. Osteoblasts are target cells for IL-1, which in turn conveys messages to osteoclasts to resorb bone.

Henneman S, Von den Hoff JW and Maltha JC (2008)[34] in his review describes the mechanical and biological signalling pathways during orthodontic tooth movement. A theoretical model is introduced to elucidate the complex cascade of events after the application of an orthodontic force to a tooth. The theoretical model describes four stages in the induction of tooth movement. These stages are as follows: (1) Matrix strain and fluid flow. Immediately after the application of an external force, strain in the matrix of the PDL and the alveolar bone results in fluid flow in both tissues. (2) Cell strain. As a result of matrix strain and fluid flow, the cells are

deformed. (3) Cell activation and differentiation. In response to the deformation, fibroblasts and osteoblasts in the PDL as well as osteocytes in the bone are activated. (4) Remodelling. A combination of PDL remodelling and the localized apposition and resorption of alveolar bone enables the tooth to move. Increased levels of transforming growth factor- β, cathepsins B and L, and interleukin-1 beta were also found in the crevicular fluid of orthodontically moved teeth in humans and at the apposition side of rat teeth after orthodontic force application.

Ren Y and Vissink A (2008)[76] in their review aimed to evaluate studies on cytokines in the gingival crevicular fluid (GCF) during orthodontic treatment, summarizing the regulation patterns of the most commonly studied cytokines and exploring their clinical implications. To achieve this, a number of key databases were searched using MESH terms and free text terms. An additional search was made by reference tracking. The procedures suggested by the QUOROM statement were followed. Data from the included studies were extracted into orthodontic mechanics, GCF sampling/handling methods, and cytokine measurements. From the 85 relevant studies identified, 23 studies could be included. Common drawbacks consisted mainly of inadequacies in the study design (e.g. short duration and small number of study subjects). The most consistent result was a peak of cytokine levels at 24 h. Associations existed between prostaglandin E_2 (PGE_2) and interleukin-1β (IL-1β) and pain, velocity of tooth movement, and treatment mechanics. Interleukin-1β and PGE_2 showed different patterns of up-regulation, with IL-1β being more responsive to mechanical stress and PGE_2 more responsive to synergistic regulation of IL-1β and mechanical force. The results might be taken to support, at the cellular level, the use of light continuous forces for orthodontic treatment.

Lee TY, Lee KJ, Baik HS (2009)[50] stated that orthodontic loading led to increases in IL-1β, MMP-9, and TIMP-1 mRNA in pressure side gingiva in rats. A total of 14 male wistar rats were used with three rats as no appliance controls and another three as the sham appliance group. On the 7th and the 14th day after orthodontic loading on the maxillary left molar, four rats were sacrificed, respectively. Maxillary right first molars served as the contralateral control side. A real-time RT-PCR for the excised gingiva was performed to measure the mRNA of IL-1β, MMP-9, and TIMP-1. Results indicated that Compared with the contralateral side, IL-1β mRNA from the pressure side significantly increased on the 7th day, then decreased on the 14th day. MMP-9 and TIMP-1 mRNA showed a significant constant increase on both the 7th and the 14th day.

INTERLEUKIN 1β GENE POLYMORPHISM AND EXTERNAL APICAL ROOT RESORPTION

Rossi N, Whitcomb S, and Lindemann R (1996)[80] based on their study carried on to determine whether L-thyroxine (T4) and thyrocalcitonin (TCA) influence monocyte production of interleukin-lβ (IL-1β) and tumor necrosis factor-α (TNF-α) and to examine IL-1β and TNF-α production in monocytes from a group of orthodontic patients with severe root shortening, stated that patient monocytes did not differ from control monocytes in regard to these cytokine parameters, and therefore in vitro IL-1β and TNF-α levels could not distinguish resorption subjects.

Al-Qawasmi RA, Hartsfield JK, Jr, Everett ET, PhD, Flury L, Liu L, Foroud TM, Macri JV and Roberts WE (2003)[3] examined Linkage and association were examined between polymorphisms of the interleukin IL-1 (*IL-1A* and *IL-1B*) genes and EARR in 35 white American families. Buccal swab cells were collected for DNA isolation and analysis. The EARR in the maxillary central incisors, the mandibular central incisors, and the mesial and distal roots of the mandibular first molar were analyzed separately and together by using both linkage and association methods of analysis. Highly significant ($P < .0003$) evidence of linkage disequilibrium of *IL-1B* polymorphism with the clinical manifestation of EARR was obtained. The analysis indicates that the *IL-1B* polymorphism accounts for 15% of the total variation of maxillary incisor EARR. Persons homozygous for the *IL-1B* allele 1 have a 5.6 fold (95% CI 1.9-21.2) increased risk of EARR greater than 2 mm as compared with those who are not homozygous for the *IL-1*β allele 1. Data indicate that allele 1 at the *IL-1B* gene, known to decrease the production of IL-1 cytokine in vivo, significantly increases the risk of EARR. Defining genetic contributions to EARR is an important factor in understanding the contribution of environmental factors, such as habits and therapeutic biomechanics.

Yamaguchi M, Ozawa Y, Mishima H, Aihara N, Kojima T, and Kasai K (2008)[111] obtained HDPF from 5 patients with severe apical root resorption after orthodontic treatment. The levels of interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α were determined after 24 hours by using ELISA kits. Furthermore, culture supernatants were added to cultured human osteoclasts, and osteoclast formation was observed after tartrate-resistant acid phosphatase (TRAP) staining and the formation of resorption cavities. Stimulation with SP increased the levels of IL-1β,

IL-6, andTNF-α, in a time- and concentration-dependent manner, although the increase was greater in the severe root resorption (SRR) group than in the nonresorption (NR) groupAs for osteoclast formation, the numbers of TRAP-positive multinucleate cells and resorptive pits were significantly increased in the SRR group compared with the NR group. These results suggest that HDPF stimulated with SP might be deeply involved in the progress of inflammation in pulp tissue and the incidence of SRR during orthodontic treatment.

Lages EMB, Drummond AF, Pretti H, Costa FO, Lages EJP, Gontijo AI, Cota LM, and Brito RB (2009)[49] showed that the polymorphism of the IL-1β gene is associated with root resorption in the studied population. Interleukin 1ß (IL-1ß) is a potent stimulus for bone resorption and osteoclastic cell recruitment during orthodontic tooth movement. The sample included 61 Brazilian orthodontic patients, divided into 2 groups according to the presence (affected group, n = 23) or absence (control group, n = 38) of EARR in the central and lateral maxillary incisors in the posttreatment period. DNA was obtained from buccal swab cells. The polymorphism was analyzed by the polymerase chain reaction followed by digestion with restriction enzyme. The polymerase chain reaction products were analyzed in 10% polyacrylamide gel and stained with silver. There were significant statistical differences among the frequencies of the alleles and genotypes of the IL-1b gene polymorphism between the affected and unaffected groups, suggesting that allele 1 predisposed the subjects to EARR.

ASSESMENT OF GCF DURING ORTHODONTIC TOOTH MOVEMENT

SIGNIFICANCE OF USING GCF FOR ASSESMENT

Krishnan V and Davidovitch Z (2006)[45] stated that GCF arises at the gingival margin and can variously be described as a transudate or an exudate. Its rate of flow is related to the degree of gingival inflammation, and a rate of 0.05 to 0.20 μl per minute was reported in cases of apparent minimal inflammation. The total fluid flow is between 0.5 and 2.4 ml per day. Recent studies in orthodontic tooth movement have used GCF because of its noninvasive nature and ease of repetitive sampling from the same site with the help of platinum loops, filter paper strips, gingival washings, and micro pipettes. The fluid is used to analyze various biochemical markers such as prostaglandin production and the action of various extracellular and intracellular factors, such as IL-1, IL-6, TNF-α, epidermal growth factors, β2 microglobulin, cathepsin, aspartate aminotransferease, alkaline phosphatase, and lactate dehydrogenase.

METHODS FOR COLLECTING GCF SAMPLES

Waddington RJ, Embery G (2001)[109] stated that GCF can be collected by variety of procedures providing noninvasive, site specific process. Its collection requires patience in part of clinician and ranges from use of platinum loops, filter paper strips, gingival washings and use of micropippetes. The author preferred the use of micropippetes because it has the advantage of ready storage and quantification.

Kavadia-Tsatala S (2002)[42] described various methods for collecting GCF samples. For collection of predetermined volumes, microcapillary tubules are placed at the gingival crevice and either held at a particular site, or passed back and forth for 10- to 15- minute periods. This procedure can often be disruptive to the delicate crevicular epithelium, resulting in contamination of the native GCF with blood and serum. Collection of significant predetermined volumes can also cause an influx of serum from the gingival capillaries, leading to a dilution of the native GCF by serum, since the usual volume range in the undisturbed sulcus is between 0.5 and 1 μL.13 GCF can also be collected by placing a prewashed absorbent string into the gingival crevice. This method is also disruptive for the epithelium and can involve problems with the accurate weighing of small samples. Another common method is the placement of filter paper strips in the gingival crevice. Sample collection may last for a specific or an indeterminate period of time. Alternatively, the initial GCF sample can be discarded and the subsequent flow sample collected. Collecting the initial GCF is less disturbing to the crevicular epithelium and enables more rapid measurements of the GCF, thus decreasing the probability of altering the GCF by excessive contamination with serum. A paramagnetic bead method has been developed as an alternative procedure for such GCF components as tumor necrosis factor (TNF).

EFFECT OF ORTHODONTIC TOOTH MOVEMENT ON GINGIVAL CREVICULAR FLUID VOLUME

A. Dannan, MA. Darwish, MN. Sawan (2012)[4] demonstrated that Orthodontic tooth movement, namely canine retraction, does not significantly increase the volume of GCF exudate. Fourteen upper and lower canines of patients with different Angle

classifications were selected for the study. After extraction of the first premolars, the canines were subjected to orthodontic distal retraction. GCF was sampled from mesial and distal gingival crevices of each canine separately at baseline, 1 hour, 7 days, 14 days, 21 days, and 28 days after the application of the orthodontic distal retraction. GCF volume was determined by means of an electronic device. GCF volume at tension sites was slightly greater after 21 and 28 days compared to other observation time points. At pressure sites, GCF volume was slightly greater after 28 days compared to other observation time points. None of the observed differences, however, was statistically significant The slight increase in GCF volume could be due to a slight degree of gingival inflammation.

ASSESMENT OF INTERLEUKIN 1 BETA IN GCF

Grieve WG, Johnson GK, Moore RN, Reinhardt RA, and DuBois LM (1994)[29] examined gingival crevicular fluid (GCF) levels of two potent bone resorbing mediators, prostaglandin E (PGE) and interleukin-1β (IL-1β), during human orthodontic tooth movement. The study included 10 patients, each having one treatment tooth undergoing orthodontic movement and a contralateral control tooth. The GCF was sampled at control sites and treatment (compression) sites before activation and at 1, 24, 48, and 168 hours. Prevention of plaque-induced inflammation allowed this study to focus on the dynamics of mechanically stimulated PGE and IL-1β GCF levels. The PGE and IL-1β levels were determined with radioimmunoassay. At 1 and 24 hours, mean GCF IL-1β levels were significantly elevated at treatment teeth (8.9 +/- 2.0 and 19.2 +/- 6.0 pg, respectively) compared with control teeth (2.0 +/-1.1

pg, p = 0.0049, and 2.9 +/- 1.0 pg, p = 0.0209, respectively). The GCF levels of PGE for the treatment teeth were significantly higher at 24 and 48 hours (108.9 +/- 11.9 and 97.9 +/- 7.3 pg) than the control teeth (61.8 +/- 7.2 pg, p = 0.0071, and 70.8 +/- 7.4 pg, p = 0.0021, respectively). The GCF levels of PGE and IL-1β remained at baseline levels throughout the study for the control teeth, whereas significant elevations from baseline in GCF IL-1β (24 hours) and PGE levels (24 and 48 hours) were observed over time in the treatment teeth ($p < 0.05$). These results demonstrate that bone-resorbing PGE and IL-1β produced within the periodontium are detectable in GCF during the early phases of tooth movement and return to baseline within 7 days.

Uematsu S, Mogi M, Deguchi T (1996)[107] in their study quantified various cytokines in human gingival crevicular fluid (GCF), and investigated the changes in their levels during orthodontic tooth movement. Twelve patients (mean age, 14.4 years) were used as subjects. An upper canine of each patient having one treatment for distal movement served as the experimental tooth, whereas the contralateral and antagonistic canines were used as controls. The GCF around the experimental and the two control teeth was taken from each subject immediately before activation, and at 1, 24, and 168 hr after the initiation of tooth movement. Cytokine levels were determined by ELISAs. They found that the concentrations of interleukin (IL)-1 beta, IL-6, tumor necrosis factor-alpha, epidermal growth factor, and beta 2-microglobulin were significantly higher in the experimental group than in the controls at 24 hr after the experiment was initiated.

Tzannetou S, Efstratiadis S, Nicolay O, Grbic J, and Lamster I (1998) [102] examined whether the inflammatory mediators interleukin (IL-1b) and b-glucuronidase (bG) are present in the gingival crevicular fluid (GCF) of children undergoing rapid

palatal expansion and whether their levels vary upon activation of the appliance and movement of the maxillary first molars. Nine adolescent patients who needed palatal expansion were studied. Each patient received a periodontal prophylaxis and instruction in proper home care, including rinsing with chlorhexidine. Four weeks later, a modified Hyrax appliance was inserted. The jackscrew was activated twice daily until the appropriate expansion was achieved. GCF samples were collected at 2 pretreatment observation periods and 9 observation periods after placement of the appliance. Samples were collected with filter paper strips and analyzed by means of ELISA and time-dependent fluorometry for IL-1b and bG, respectively. The values recorded at the observation period 2 weeks after the periodontal prophylaxis were used as baseline. The results indicate that (1) bG and IL-1b are present in GCF of young, healthy individuals, (2) their levels decrease following a strict regimen of plaque control, (3) orthodontic/orthopedic forces evoke changes in the levels of the inflammatory mediators IL-1b and bG in the periodontal tissues that can be detected in GCF. The results of this study support the hypothesis that mechanical stimulus causes an inflammatory reaction within the periodontal tissues, which in turn may trigger the biological processes associated with bone remodeling

Lee KJ, Park YC, Yu HS, Choi SH, and Yoo YJ (2004)[51] showed that the analysis of crevicular fluid is a useful method for assessing cellular response to orthodontic force in vivo. Ten healthy young adults (mean age 20.6 years, 2 men, 8 women) with 4 premolars extracted were assessed. In each subject, 1 maxillary canine (E1) received continuous force with a nickel-titanium coil spring. The opposite canine (E2) received an interrupted force with a screw-attached retractor; the force was reactivated weekly by 2 turns of the screw. An antagonistic canine was used as a

control. Gingival crevicular fluid was collected from the distal side of each tooth, 10 times in 3 weeks, and IL-1β and PGE2 levels were measured. For E1, the IL-1β level showed a significant elevation at 24 hours and then decreased and maintained an insignificant but high mean concentration, compared with the control site. The PGE2 level showed a significant elevation at 24 hours and then decreased. For E2, a significant elevation of IL-1β level was observed at 24 hours and a greater significant elevation at 24 hours after the first reactivation, compared with the control sites. The PGE2 level increased significantly at 24 hours and remained high for 1 week. The synergistic up-regulation of PGE2 by appliance reactivation and secreted IL-1β was not evident with either type of force after 1 week. Both experimental sites showed significant tooth movement compared with the control sites at 3 weeks; however, there was no significant difference between the 2 experimental sites. A well-controlled mechanical stress with timely reactivation can effectively upregulate IL-1β secretion, but there might be limitations in increasing the mediator levels, because of the feedback mechanisms in vivo.

Iwasaki LR, Crouch LD, Tutor A, Gibson S, Hukmani N, Marx DB, and Nickela JC (2005)[38] stated that Interindividual differences in cytokine concentrations measured in stimulated whole blood (SWB) correlated with the velocity of tooth translation. Continuous maxillary canine retraction stresses of 13 kPa and 4, 26, or 52 kPa were applied bilaterally in 6 growing and 4 adult subjects for 84 days. Dental models and GCF samples were collected at 1- to 14-day intervals. Cytokines were measured in GCF and SWB cell cultures. Velocity of tooth translation (*v*t) varied with growth status and stresses $\leq$ 52 kPa; stresses of < 52 kPa showed no lag phase; and

equivalent stresses yielded subject-dependent differences in *v*t, which correlated with cytokines in GCF and SWB.

Yamaguchi M , Yoshii M and Kasai K (2005)[112] stated that metabolism by peptidases plays an important role in modulating the levels of biologically-active neuropeptides, while that of substance P (SP), a component of gingival crevicular fl uid (GCF), may potentiate the inflammatory process in orthodontic tooth movement. The subjects assesed were 3 males, with a mean age of 21.3 ± 2.8 years old, and 6 females, with a mean age of 23.1 ± 2.4 years, undergoing orthodontic movement of a single tooth, with the contralateral tooth used as the control. GCF was sampled at the control and treatment (compression) sites before and 1, 4, 8, 24, 72, 120, and 168 hours after initiation of orthodontic treatment. Prevention of plaque-induced inflammation allowed assessment of the dynamics of mechanically stimulated SP and IL-1 β levels in the GCF, which were determined using enzyme-linked immunosorbent assay (ELISA) kits. GCF levels of SP and IL-1 β for the treated teeth were signifi cantly higher ($P < 0.001$) than for the corresponding control teeth from 8 to 72 hours, and peaked at 24 hours. These results show that the amounts of SP and IL-1 β in GCF increase with orthodontic tooth movement, and indicate that such increases may be involved in inflammation in response to mechanical stress.

Iwasaki LR, Gibson CS, Crouch LD, Marx DB, Pandey JP, and Nickela JC (2006)[39] based on their study stated that speed of tooth movement is relayed to stress and interleukin 1 gene polymorphisms. Ten subjects had their maxillary first premolars extracted and cheek wipe samples genotyped. In each subject, a maxillary canine received 26 kPa and the other received 13 or 52 kPa of stress. GCF samples and tooth movements were measured at 9 or 10 visits over 84 days. Faster velocity of tooth

translation was shown from 26 kPa than 13 kPa ($P < .015$) and 52 kPa ($P < .030$), with higher IL-1β/IL-1RA in GCF at experimental relative to control sites.

Dudic A, Kiliaridis S, Mombelli A and Giannopoulou C (2006)[19] evaluated whether the application of tension or compression forces exerted on the periodontium during the early phase of orthodontic tooth movement is reflected by differences in the composition of the gingival crevicular fluid (GCF), at the level of interleukin-1β (IL-1β), substance P (SP), and prostaglandin E_2 (PGE_2). Eighteen children (mean age 10.8 yr) starting orthodontic treatment were included in the study. Molar elastic separators were inserted mesially to two first upper or lower molars. One of the antagonist molars served as the control. GCF was collected from the mesial and distal sites of each molar, before (−7 d, 0 d) and immediately after (1 min, 1 h, 1 d, and 7 d) the placement of separators. The levels of IL-1β, SP, and PGE_2 were determined by enzme-linked immunosorbent assay. At the orthodontically moved teeth, the GCF levels of IL-1β, SP, and PGE_2 were significantly higher than at the control teeth in both tension and compression sides, and at almost all occasions after insertion of separators. The increase, relative to baseline values, was generally higher in tension sides. For the control teeth, the three mediators remained at baseline levels throughout the experiment. The results suggest that IL-1β, SP, and PGE_2 levels in the GCF reflect the biologic activity in the periodontium during orthodontic tooth movement.

Basaran G, O¨zer T, Kaya FA, Kaplan A, Hamamcı O (2006)[7] argued that all the studies that determined the level of cytokines in gingival crevicular fluid (GCF) evaluated subjects, both animals and humans, for short periods of time. These studies applied distalization forces to the teeth and searched for early responses to the forces. As a result of the great force that is applied to the tooth, a hyalinized zone occurs in the

compressed periodontal ligament. This hyaline zone has also been described as an area of focal aseptic necrosis. From the tension side, an indirect resorption process initiates, which regulates bone resorption. But orthodontic treatment does not always use distalization forces that produce hyalinization as is considered in these studies. All the preceding studies applied heavy distalization forces on tooth. As a result of this pressure on the periodontium, hyaline zones occurred and the indirect resorption process initiated and induced levels of IL- 1β and TNF-α. However, in the first stages of orthodontic treatment, low forces are used that do not result in hyalinized zones and indirect resorption. Therefore, there may be different levels of cytokines in the leveling and distalization stages of the orthodontic treatment. They did a study to test whether interleukine 1β (IL-1β) and tumor necrosis factor–α (TNF-α) levels differ from each other in different treatment levels. Eighteen patients, nine female and nine male (aged 16–19 years; mean 17.4 +/- 1.8 years), participated in this study. The patients were seen at baseline, at days 7 and 21 and at the 3rd and 6th month as the leveling of the teeth occurred. Records of the baseline scores for the distalization forces were taken at the 6th month. Days 7 and 21 after 6 months of treatment were also recorded. Results suggested that there were increases in the volume of gingival crevicular fluid (GCF) and in the concentrations of IL-1β and TNF-α. They concluded by stating that leveling and distalization of the teeth evoke increases in both the IL-1β and TNF-αlevels that can be detected in GCF.

Tzannetou S, Efstratiadis S, Nicolay O, Grbi J, and Lamster I (2008)[103] studied nine patients requiring palatal expansion. Each patient received periodontal prophylaxis and instructions in proper home care including rinsing with chlorhexidine.

Four weeks after periodontal prophylaxis, a modified hyrax appliance was placed. The jackscrew was activated twice daily until the appropriate expansion was achieved. GCF samples were collected before and after periodontal prophylaxis and during passive wearing of the appliance, active orthodontic treatment, and retention. Fluid samples were collected with filter paper strips and analyzed by ELISA and time-dependent fluorometry for IL-1β and βG, respectively. The values recorded after periodontal prophylaxis were used as the baseline. The results validate that IL-1β and βG are present in the GCF of adolescents, and, although their level decreases after a strict regimen of plaque control, it increases during orthodontic or orthopedic movement. Moreover, this study demonstrates that both heavy and light forces evoke increased levels of IL-1β and βG, stronger forces cause higher levels of inflammatory mediators, and both IL-1β and βG respond to direct and indirect application of mechanical force to teeth.

Giannopoulou C, Mombelli A, Tsinidou K, Vasdekis V, and Kamma J (2008)[24] studied the expression of IL-1β, IL-4, and IL-8 in the gingival crevicular fluid (GCF) of children, adolescents, and young adults with and without fixed orthodontic appliances. Their findings suggested that fixed orthodontic appliances result in an increase in the expression of IL-1β and IL-8. This may reflect biologic activity in the periodontium during orthodontic tooth movement.

Luppanapornlarp S, Kajii TS, Surarit R and Iida J (2010)[56] assessed interleukin (IL)-1b levels in human gingival crevicular fluid (GCF), pain intensity, and the amount of tooth movement during canine retraction using different magnitudes of continuous orthodontic force. The upper canines were retracted with continuous forces of 50 or 150 g using nickel–titanium coil springs on segmented archwires. No

significant difference in the amount of tooth movement was found between these two different magnitudes of continuous force at 2 months. A 50 g force could effectively induce tooth movement similar to 150 g with less pain and less inflammation.

Kaya FA, Hamamci N, Basaran G, Dogru M, and Yildirim TT (2010) [44] determined tumor necrosis factor alpha (TNF-α), interleukin 1β (IL-1β) and interleukin 8 (IL-8) levels in GCF in tooth early levelling movement orthodontic treatment in seventeen patients, 8 female and 9 male individuals (aged: 16-20 years; mean 18.2 ± 1.4 years). Orthodontic forced induces rapid release of the TNF-α, 1L-1β and IL 8 levels during tooth movement in gingival crevicular fluid (GCF). The results of this study support the hypothesis that proinflammatory cytokines play a potent role in bone resorption after the application of orthodontic force in short time.

Shetty SK, Kumar M, and Smitha. P. L. (2011)[98] in their review article evaluated cytokines in the gingival crevicular fluid (GCF) during orthodontic treatment. They stated that Association exists between Prostaglandin E2 (PGE2) and Interleukin-(1L-1β) and pain, velocity of the tooth movement and treatment mechanics. IL-1β and PGE 2 showed different patterns of upregulation with IL-1β being more responsive to mechanical stress and PGE 2 more responsive to synergistic regulation of IL-1β and mechanical force. The results might be taken to support, at the cellular level, the use of light continuous forces for orthodontic treatment.

Kaya FA, Arslan SG, Kaya CA, Arslan H, and Hamamci O (2011)[43] demonstrated that the cytokine levels of orthodontic force applied teeth in late adult rats are compatible with the levels of studies in young rats. In experiment 19 adult (120 days) Spraque-Dawley rats were used. Approximately 15 g force applying open coil spring was applied actively between the upper incisors of the rats. Before and after the

activation on the 3rd and 7th and 10th days GCF samples were taken from the vestibular surfaces of appliance fixed teeth using periopaper®. Then the samples were biochemically analyzed. For the statistical analysis of working days of each cytokines repetitive variance analysis technique was used. Results indicated that the levels of IL-1β, IL-6 and TNF-α were the highest in the 3rd day and started to decrease on the 7th and 10th days.

Gong Y, Lu J, and Ding X (2011)[25] investigated the microbiologic and immunologic factors related to orthodontic treatment-induced gingival enlargement (GE). Study included 12 patients with GE undergoing fixed orthodontic treatment and 12 periodontally healthy controls. At baseline, periodontal variables, subgingival plaque samples, and gingival crevicular fluid (GCF) samples were taken from 2 preselected sites in both the GE and the control groups. The levels of Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, Prevotella intermedia, Treponema denticola and Tannerella forsythia were determined by real-time polymerase chain reaction. GCF interleukin (IL)-1b and transforming growth factor-beta 1 (TGF-b1) were detected by enzyme-linked immunosorbent assay (Invitrogen, Camarillo, Calif). Periodontal therapy was given to the GE group, and all parameters were reassessed after 4 weeks. IL-1b and TGFb1 levels at the GE sites were also significantly higher than those at the control sites. They concluded by stating that Periodontal pathogens might have a relationship with the initiation and development of orthodontic treatment-induced GE. Inflammatory cytokines (IL-1b and TGF-b1) can also be considered as contributing factors.

ASSESMENT OF OTHER MARKERS IN GCF

Michael I, Gregory JK, DMD, and Stephen DK (1996)[61] demonstrated that phosphatase activities in GCF may be a useful means for monitoring tissue responses to orthodontic treatment. Three female subjects were observed longitudinally to assess tooth movement, plaque, and inflammation. For each subject, one randomly selected premolar served as the control and was not treated, and another was moved buccally with 100 gm of force. The GCF was collected weekly and assayed for phosphatases. Alkaline phosphatase peaked between the first and third weeks, followed by an increase in acid phosphatase between the third and sixth weeks.

Perinetti G, Paolantonio M, D'Attilio M ,D'Archivio D, Tripodi D, Femminella B, Festa F, and Spoto G (2002)[70] assessed alkaline phosphatase (ALP) activity in gingival crevicular fluid (GCF). Sixteen patients (mean age, 15.5 years) participated in the study. The maxillary first molars under treatment served as the test teeth (TT) in each patient; in particular, 1 first molar was to be retracted and hence was considered the distalized molar (DM), whereas the contralateral molar (CM) was included in the fixed orthodontic appliance but was not subjected to the distal forces. The DM antagonist first molar (AM), free from any orthodontic appliance, was used as the baseline control. GCF ALP activity was significantly elevated in the DMs and the CMs as compared with the AMs at 1, 2, 3, and 4 weeks; conversely, in the AMs, GCF ALP activity remained at baseline levels throughout the experiment. Moreover, the enzyme activity in the DMs was significantly greater than in the CMs. In the DMs, a significantly greater ALP activity was observed in sites of tension compared with sites of compression. This difference was not seen with the CMs, in which the enzyme

activity increased to the same extent in tension and compression sites. These results suggest that ALP activity in GCF reflects the biologic activity in the periodontium during orthodontic movement and therefore should be further investigated as a diagnostic tool for monitoring orthodontic tooth movement in clinical practice.

Emanuela S, Giuseppe P, Michele D, Chiara C, Michele P, Felice F, and Giuseppe Spoto (2003)[20] indicated a possible role of GCF LDH during the early phases of orthodontic treatment. Thirty-seven subjects, 16 males and 21 females (mean age 18.7 years, range 14.0 to 26.7 years), participated in this study. Each subject underwent a session of professional oral hygiene and received oral hygiene instructions; 2 weeks later, a fixed orthodontic appliance was placed on the maxillary arch. A randomly selected maxillary canine was considered as the test tooth, and its antagonist, which had no appliance, was used as the control tooth. From 2 to 12 weeks after orthodontic appliance placement, GCF was harvested from both experimental teeth at the mesiobuccal angle, for GCF volume and LDH activity determinations. GCF LDH activity in the test teeth was significantly greater than that of the control teeth.

Tuncer BB, O¨ zmeric N, Tuncer C, Teoman I, Yu cel BA, Alpar R, Balos K (2005)[101] evaluated the levels of IL-8 during mechanical forces on periodontal tissues at different stages of orthodontic therapy. Ten canine teeth of patients having different Angle classifications were selected for the study. After the premolars were extracted, the maxillary/mandibular canines were tipped distally. Gingival crevicular fluid was sampled from mesial and distal gingival crevices of each canine separately at baseline and one hour, 24 hours, six days, 10 days, and 30 days after the application of the force. An enzyme-linked immunosorbent assay for quantitative detection of IL-8 was used. Although there was an increase in the concentration of IL-8 at tension (mesial) sites

after one hour, 24 hours, six days, and 10 days, a decrease was observed at 30 days. Pressure (distal) sites did not demonstrate such an increase at any period except at 10 days. However, the concentration of IL-8 at both sites showed a similar decrease and approached each other at day 30. They concluded that local host response toward the orthodontic forces might lead an increase in IL-8 and neutrophil accumulation, and this may be one of the triggers for bone remodeling processes.

Basaran G, Özer T, Kaya FA, and Hamamci O (2006)[6] determined levels of interleukins 2, 6, and 8 during tooth movement. Fifteen patients (9 female, 6 male; ages, 15-19 years; mean age, 16.7 +/- 2.3 years) participated in this study. Each underwent a session of professional oral hygiene and received oral hygiene instructions. Two months later, a fixed orthodontic appliance was placed. The patients were seen at baseline, at days 7 and 21, and as the teeth were leveled. Records of the baseline scores for the distalization forces were taken at the sixth month. Scores of days 7 and 21 after 6 months of the distalization treatment were also recorded. Increases were seen in the volume of gingival crevicular fluid and the concentrations of interleukins 2, 6, and 8. Leveling and distalization of the teeth evoke increases in interleukins 2, 6, and 8 levels in the periodontal tissues that can be detected in gingival crevicular fluid.

Sari E, Kadioglu O, Ucar C and Altug HA (2010)[92] compared Prostaglandin E2 (PGE2) levels in gingival crevicular fluid (GCF) of young adults with maxillary constriction during tooth- and bone-borne expansion. Thirty patients, 15 females and 15 males, with a mean age of 17.3 ± 2.8 years were divided into three groups. Group I consisted of 10 patients, five females and five males, treated by transpalatal distraction (TPD) as a bone-borne device, group II 10 patients, five females and five males, with a

Hyrax appliance as a tooth-borne device, and a control group of 10 patients, five females and five males, without any expansion appliances. The mean PGE2 level was significantly elevated on day 4 after placement of the separators in group II ($P < 0.05$). The PGE2 values in group II were significantly different to those in group I and the controls at all observation periods. Lower PGE2 levels were observed in group I compared with group II and the controls. Expansion using the TPD method could potentially enhance the prognosis of the teeth by inducing more skeletal dental changes when compared with the Hyrax appliance.

Gastel JV, Teughel W, Quirynen M, Struyf S, Damme JV, Coucke W, and Carelsg C (2011)[21] showed that Interleukin-6 and interleukin-8 concentrations before orthodontic treatment were shown to be significant predictive factors for some potential inflammatory parameters during treatment. This longitudinal split-mouth trial included 24 patients. Supragingival and subgingival plaque composition, probing depth, bleeding on probing, and GCF flow and composition were assessed at baseline (Tb) and after 1 year (T52). Between Tb and T52, the pathogenicity of the plaque and all periodontal parameters increased significantly, but intersite differences were not seen, except for bleeding on probing. The cytokine concentrations in the GCF did not differ significantly between the sites or between Tb and T52. The interleukin-6 concentration in the GCF at Tb was a significant predictive value for the GCF flow at T52 ($P<0.05$). The same relationship was found between the interleukin-8 concentration at Tb and the increase in probing depth at T52 ($P<0.05$).

Alfaqeeh SA and Anil S (2011)[1] showed that cross-linked N-telopeptides of type I collagen marker (NTx) and osteocalcin (OC) levels can be successfully estimated in the GCF, and its increased levels might indicate the active tooth

movement phase in orthodontic therapy. Twenty patients requiring all first premolars to be extracted were selected and treated with conventional straight-wire mechanotherapy. The canines were retracted with closed-coil springs. GCF was collected from around the canines before retraction, and 1 hour, 1 day, 7 days, 14 days, and 21 days after retraction, The results showed statistically significant changes in NTx and OC levels on days 7, 14, and 21 when we compared the experimental and control sides. The peak in all activity of the variables occurred on day 14 after retraction.

ASSESMENT OF SALIVA DURING BONE REMODELLING

SIGNIFICANCE OF USING SALIVA FOR ASSESMENT

Kaufman E and Lamster IB (2002)[40] in their review examined the diagnostic application of saliva for systemic diseases. As a diagnostic fluid, saliva offers distinctive advantages over serum because it can be collected non-invasively by individuals with modest training. Furthermore, saliva may provide a cost-effective approach for the screening of large populations. Gland-specific saliva can be used for diagnosis of pathology specific to one of the major salivary glands. Whole saliva, however, is most frequently used for diagnosis of systemic diseases, since it is readily collected and contains serum constituents. These constituents are derived from the local vasculature of the salivary glands and also reach the oral cavity *via* the flow of gingival fluid.

Nomura Y, Tamaki Y, Tanaka T, Arakawa H, Tsurumoto A, Kirimura K, Sato T, Hanada N, Kamoi K (2006)[67] stated that gingival crevicular fluid might be

a potential candidate for screening of periodontitis. However, there are different sampling sites in oral cavity and the differences in sites must be considered. In addition the sampling technique is not easy and a long time is needed for collection of sample. Saliva contains many enzymes and some inflammatory markers and no specific laboratory devices are needed for collection of saliva.

Pfaffe T, Cooper-White J, Beyerlein P, Kostner K, and Punyadeera C (2011)[71] stated that Over the past 10 years, the use of saliva as a diagnostic fluid has gained attention and has become a translational research success story. As a diagnostic fluid, saliva offers advantages over serum because it can be collected noninvasively by individuals with modest training, and it offers a cost effective approach for the screening of large populations. There is minimal risk of contracting infections during saliva collection, and saliva can be used in clinically challenging situations, such as obtaining samples from children or handicapped or anxious patients, in whom blood sampling could be a difficult act to perform. Saliva harbors a wide spectrum of proteins/peptides, nucleic acids, electrolytes, and hormones that originate from multiple local and systemic sources.

METHODS OF COLLECTIONG SALIVA SAMPLE

Pfaffe T, Cooper-White J, Beyerlein P, Kostner K, and Punyadeera C (2011)[71] in their review article described various methods for collecting saliva. Saliva can be collected under unstimulated (resting) or stimulated conditions. In brief, whole mouth resting saliva can be collected by the draining/ drooling method, the spitting method, the swabbing method, and the suction method. Stimulated saliva is collected

by either having the patient chew a piece of paraffin and/or by applying 0.1– 0.2 mol/L (approximately 1 drop) citric acid to the tongue. In addition, saliva can be probed from individual glands by using cannulation of the glandular ducts or by the application of specific collecting devices to the emergence area of the glandular ducts. At present, there are companies that manufacture commercial saliva collection devices for diagnostic and research purposes. These include: Salimetrics oral swabs (http://www.salimetrics.com); Oasis Diagnostics® VerOFy® I/II; DNA_SAL™ (http:// www.4saliva.com); OraSure Technologies OraSure HIV specimen collection device (http://www.orasure.com); CoZart® drugs of abuse collection devices (http://www.concateno.com); and the Greiner Bio- One Saliva Collection System (http://www.gbo.com).

ASSESMENT OF INTERLEUKIN 1 BETA IN SALIVA DURING PERIODONTITIS – BONE REMODELLING

Mogi M, Otogoto J, Ota N, Inagaki H, Minami M, Kojima K (1999)[63] collected GCF samples from patients with periodontal disease and from controls. The concentrations of epidermal growth factor, transforming growth factor (TGF)-alpha, interleukin (IL)-1 beta, IL-6, interferon-gamma, beta 2-microglobulin (beta 2-MG), and apoptosis-related modifiers sFas and bcl-2 in the samples were determined by enzyme-linked immunosorbent assay. TGF-alpha was significantly lower in patients with periodontal disease than in the controls. In contrast, the concentrations of IL-1 beta, IL-6; and beta 2-MG were significantly higher in the group with severe periodontal disease than in the controls.

R. P. Teles, V. Likhari, S. S. Socransky, and A. D. Haffajee (2009) [81] in their cross-sectional study, periodontally examined 74 subjects with chronic periodontitis and 44 periodontally healthy individuals and had the levels of granulocyte–macrophage colony-stimulating factor, interleukin-1β, interleukin-2, interleukin-4, interleukin-5, interleukin-6, interleukin-8, interleukin-10, interferon-γ and tumor necrosis factor-α measured in whole saliva using a multiplexed bead immunoassay (Luminex). Based on their results they concluded that mean salivary levels of granulocyte–macrophage colony-stimulating factor, interleukin-1β, interleukin-2, interleukin-4, interleukin-5, interleukin-6, interleukin-8, interleukin-10, interferon-γ and tumor necrosis factor-α could not discriminate between periodontal health and disease.

Kaushik R, Yeltiwar RK, and Pushpanshu K (2011) [41] assessed and compared the salivary IL-1β levels in patients with chronic periodontitis before and after periodontal phase I therapy and periodontally healthy controls. Twenty-eight patients with moderate-to-severe generalized chronic periodontitis and 24 age-, race-, and ethnicity-matched controls participated in this study. Saliva samples were obtained from all patients. The clinical parameters recorded were clinical attachment loss (AL), probing depth, bleeding on probing, periodontal index, and gingival index. Clinical evaluation and sample collection were repeated 1 month after periodontal phase I therapy in patients with periodontitis. IL-1β levels were assessed using enzyme-linked immunosorbent assay. Mean IL-1β levels in patients with periodontitis at baseline (1,312.75 pg/mL) were significantly higher (P <0.0001; eight-fold) than in controls (161.51 pg/mL). Although treatment in patients with periodontitis resulted in significant reduction in IL-1β levels (mean: 674.34 pg/mL; P= 0.001), they remained

significantly higher (P <0.0001; four-fold) than control levels. The data indicate that IL-1β levels are raised in the saliva of patients with chronic periodontitis, which are reduced after phase I therapy, suggesting a close association between salivary IL-1β and periodontitis.

STUDIES COMPARING THE CHANGES IN INTERLEUKIN 1β IN SALIVA AND GCF DURING PERIODONTAL BREAKDOWN

Ülker EA, Tulunoglu O, Özmeric N, Can M, and Demirtas S (2008)[106] evaluated the levels of cystatin C, interleukin-1β (IL-1β), and tumor necrosis factor-alpha (TNF-α) in the total saliva and gingival crevicular fluid (GCF) of periodontally healthy children (PHC) and children with gingivitis (CG) who were between 11 and 16 years old. The study was carried out with 10 PHC and 25 CG. Unstimulated total saliva and GCF samples were obtained. After sampling, biochemical analyses were performed using latex particle–enhanced turbidimetric immunoassay for cystatin C and enzyme-linked immunosorbent assay for IL-1β and TNF-α. In total saliva, cystatin C and TNF-α levels were higher in PHC, and IL-1β levels were higher in CG, but the differences were not statistically significant. In GCF, cystatin C levels were higher in PHC, whereas TNF-α and IL-1β levels were higher in CG. The results showed that total saliva and GCF cystatin C levels were higher in PHC, but there was no correlation between cystatin C levels and IL-1β or TNF-α levels in total saliva or GCF.

STUDIES COMPARING THE CHANGES IN BIOMARKERS IN SALIVA AND GCF DURING ORTHODONTIC TOOTH MOVEMENT

Burke JC, Evans CA, Crosby TA, and Mednieks MI (2002)[9] measured Total secretory proteins and a cyclic adenosine monophosphate (AMP)-dependent protein kinase subunit (RII) in saliva and gingival crevicular fluid (GCF) after the placement of orthodontic separators to determine if mechanical force applied to teeth affects protein secretion. Whole saliva and GCF were collected before and 1 day after treatment. Electrophoresis and Western blotting were carried out to establish the banding patterns of total proteins and to measure the isotype and amount of RII that serves as an apparent stress indicator. Digitized image files were used for densitometric analyses of the relative concentrations of RII and total protein. Individual protein values showed no statistically significant changes in saliva or GCF. Western blots, however, showed a dramatic difference in RII after the placement of separators: the 50-to-55 kilodaltons (kd) band virtually disappeared and was replaced by a fragment in the 20-kd range. These results suggest that although the expression of total proteins is not altered by mechanical force applied to teeth, a systemic response via the cyclic AMP signaling pathway might have been activated.

Marcaccini AM, Amato PAF, Leao FV, Gerlach RF, and Ferreira JTL (2010)[58] stated that Myeloperoxidase (MPO) is an enzyme found in polymorphonuclear neutrophil (PMN) granules, and it is used to estimate the number of PMN granules in tissues. So far, MPO has not been used to study the inflammatory alterations after the application of orthodontic tooth movement forces. The aim of this study was to determine MPO activity in the gingival crevicular fluid (GCF) and saliva

(whole stimulated saliva) of orthodontic patients at different time points after fixed appliance activation. Methods: MPO was determined in the GCF and collected by means of periopaper from the saliva of 14 patients with orthodontic fixed appliances. GCF and saliva samples were collected at baseline, 2 hours, and 7 and 14 days after application of the orthodontic force. Mean MPO activity was increased in both the GCF and saliva of orthodontic patients at 2 hours after appliance activation ($P<0.02$ for all comparisons). At 2 hours, PMN infiltration into the periodontal ligament from the orthodontic force probably results in the increased MPO level observed at this time point. They concluded by saying that MPO might be a good marker to assess inflammation in orthodontic movement and it deserves further studies in orthodontic therapy.

Materials and Methods

MATERIALS AND METHODS

The study protocol was reviewed by the Institutional Review Board (IRB) of Ragas Dental College and Hospital, Chennai and informed consent was obtained from all the subjects.

The study group consisted of 10 subjects with the following **inclusion criteria**:

1. All of them were treated by post-graduate students in the Department of Orthodontics, Ragas Dental College and Hospital, Chennai.

2. These subjects, 6 females and 4 males ranged in age from 15 yrs to 25 yrs.

3. All the patients were treated using .022 Roth Ovation fully programmed brackets.

4. All the patients had Little's Irregularity Index score referring to severe incisor crowding

5. All the subjects were indicated for upper and lower first bicuspid extraction.

6. All subjects were in good general health with healthy periodontal tissues.

7. Probing depths were < 3mm with no radiographic evidence of periodontal bone loss.

Subjects were **excluded if**:

- Patients had active signs of periodontal disease.
- They had antibiotic therapy during the previous six months

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- They had taken anti-inflammatory medication during the month preceding the start of the study.

METHODS

Little's Index Scores were assessed with the help of a hand held digital caliper (Figure 1). It was done by measuring the linear displacement of the anatomic contact points (as distinguished from the clinical contact points) of each mandibular incisor from the adjacent tooth anatomic point on the study model with the help of a digital caliper calibrated to at least tenths of a millimeter. Care was taken that the caliper was held parallel to the occlusal plane while the beaks are lined up perpendicular with the contact points to be measured to ensure that the displacement only in the horizontal direction is recorded. After five measurements were obtained from the lower anteriors, total of all measurements were done and the inference was made based on the rankings given in Table 1. Only the patients with more than 10 mm of crowding requiring first premolar extractions were included in the study.

All the subjects were bonded with fully programmed .022 Roth Ovation brackets following upper and lower first bicuspid extraction.

An .014" Niti archwire (Orthonol NiTi Preformed natural arch, RMO) was used initially for Leveling and aligning in both upper and lower arch for all the patients. All subjects received repeated oral hygiene instructions, which included the correct use of a toothbrush and an interdental brush.

The subjects were told not to eat or drink for 1 hour before the examination. Gingival crevicular fluid and whole saliva were collected at the following stages

T 0 – Pre Treatment

T 1 – 7 days after initiation of orthodontic treatment

T 2 - 30 days after inititation of orthodontic treatment

GCF collection

- To avoid contamination of the GCF samples, small deposits of plaque were removed with a periodontal probe, and heavy deposits with a sickle scaler.

- Following the isolation and drying of a site, disposable micropipettes (Ringcaps® -Hirschmann Labergerate, Hauptstr) (Figure 2) were inserted into the entrance of the gingival crevice.

- 4-5µl of GCF was collected from the six anterior teeth in mandibular arch (Figure 4).

- Sampling was performed only on the buccal sides of the tooth to prevent salivary contamination

- The collected GCF was stored in sterile Eppendorf tubes (Figure 3) at -21°C.

- Seven days after commencement of orthodontic treatme, on the second appointment, patients were instructed to brush their teeth and not to eat anything 3 hours before the second sampling. The forces that were applied on the tooth were still active and the same procedure for collecting the GCF was performed.

- At the end of 30 days, a third sampling was performed applying the same procedures.

Saliva collection

- Whole saliva was collected by asking the patient to rinse twice with water and to drink 2 to 3 ounces of water to avoid contamination with respiratory secretions.
- The patient was instructed to allow 5 ml of saliva to flow into a sterile cup without actively expectorating (Figure 5)
- The salivary samples were centrifuged for 10 minutes, and the supernatants were frozen at -21°C until analyses.
- Samples were collected before the start of the treatment (T_0), 7^{th} day after the start of orthodontic treatment (T_1) and 30^{th} day after the start of treatment (T_2) using the same procedure.

ELISA Testing for Interleukin - 1β

The collected GCF and whole saliva samples were tested for Interleukin - 1β using the HUMAN IL - 1β ELISA kit (Gen-Probe Diaclone SAS, Besançon Cedex France)(Figure 6).

Assay Procedure

1. Each of the collected samples was diluted using the sample diluents provided in the kit.

2. 100 µl of sample was added per well.

3. 50 µl of diluted biotinylated anti IL - 1β was added to each well.

4. The wells were covered with plastic plate cover and incubated at room temperature (18 to 25°C) for 3 hours

5. The cover was removed and the plate was washed as follows: -

    a) the liquid from each well was aspirated,

    b) 0.3 ml of 1x washing solution was dispensed into each well,

    c) the contents of each well was aspirated,

    d) step b and c was repeated another 2 times.

6. 100 µl of Streptavidin – HRP solution was added into all wells.

7. The wells were covered with plastic plate cover and incubated at room temperature (18 to 25°C) for 30 minutes.

8. The wash step was repeated.

9. 100 µl of ready to use TMB Substrate Solution was added into all wells.

10. It was then incubated in the dark for 10 – 20 minutes at room temperature. Direct exposure to light was avoided by wrapping the plate in aluminium foil.

11. 100 µl of H_2SO_4: Stop Reagent was added into all wells.

12. The color change was measured spectrophotometrically at a wavelength of 450 nm ± 2 nm using an ELISA plate reader (Figure 7).

TABLE 1: LITTLE'S INDEX – RANKING BASED ON SCORE

Score	Rank
0	Perfect alignment
1 – 3	Minimal irregularity
4 – 6	Moderate irregularity
7 – 9	Severe irregularity
10	Very severe irregularity

FIGURE 1: ASSESSMENT OF PRETREATMENT LITTLE'S INDEX SCORES WITH DIGITAL CALIPER

FIGURE 2: MICROPIPETTE FOR GCF COLLECTION

FIGURE 3: EPPENDORF TUBES

FIGURE 4 : CLINICAL PROCEDURE FOR GCF COLLECTION

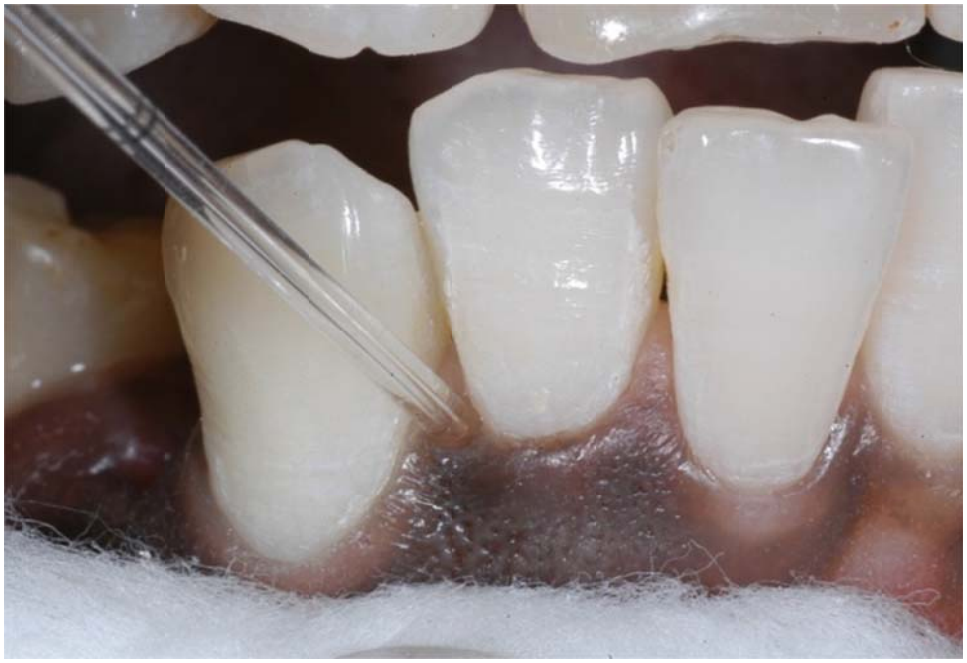


FIGURE 5: STERILE CONTAINER FOR COLLECTING SALIVA

FIGURE 6: ELISA KIT

FIGURE 7: ELISA PLATE READER

Results

RESULTS

The optical density of the samples was determined using an ELISA plate reader (Figure 6). The concentration of Interleukin - 1β (pg/ml) in the samples was then determined by comparing the optical density of the samples to the standard curve. Linear regression analyses were employed to obtain the standard curve (Figure 8) from which the corresponding concentrations of Interleukin - 1β (pg/ml) were determined (Table 2).

STATISTICAL ANALYSIS

All statistical analysis was performed by using SPSS software package (SPSS for Windows XP, version 16.0, Chicago). For each variable measured on the Saliva and GCF samples at various observation periods (T0, T1 and T2) the mean and standard deviation were calculated (Table 3).

Independent T Test was done to compare the levels of Interleukin - 1β at T0 (Pre treatment), T1 (7^{th} day after the start of orthodontic treatment) and T1 (30^{th} day after the start of orthodontic treatment) between the GCF and Saliva groups.

One way ANOVA was used to compare the mean values of Interleukin - 1β at various observation periods (T0, T1 and T2) in Saliva and GCF groups.

Tukey HSD Post Hoc tests were used for multiple pair wise comparisons within the groups for each variable.

Interpretation of P value

- The level of statistical significance was set at p=0.05.

- If the value of P > 0.05, then the inference is that it is statistically not significant.

- However, if the value of P < 0.05, then the inference is that it is statistically significant.

COMPARISON BETWEEN GCF AND SALIVA GROUPS

- The IL - 1β levels were highly increased on the 7th day in both GCF and saliva (GCF - 288.57 +/- 111.88 Pg/ml, Saliva - 272.50 +/- 108.16 Pg/ml) after appliance activation when compared to baseline levels (GCF - 151.20 +/- 129.21 Pg/ml, Saliva - 186.30 +/- 163.57 Pg/ml), and they gradually decreased on the 30th day (GCF - 228.67 +/- 103.37 Pg/ml, Saliva - 211.75 +/- 120.09 Pg/ml) when compared to the increased levels seen on 7th day (Figure 10).
- No statistically significant results were found between the two groups at all the three observation periods ($P > .05$) (Tables 4,5 and 6).

GCF LEVELS OF INTERLEUKIN - 1β AT VARIOUS OBSERVATION PERIODS

- The mean concentration of Interleukin – 1β in GCF was 151.20 +/- 129.21 Pg/ml at T0, 288.57 +/- 111.88 Pg/ml at T1, and 228.67 +/- 103.37 Pg/ml at T2 (Figure 8).
- The baseline levels for the concentration of IL-1β increased on day 7 after the start of orthodontic treatment and tended to decrease on day 30 after the start of orthodontic treatment when compared to the increased levels seen on day 7.
- Individual variations were very great.
- No statistically significant results were found between the groups because of large amout of inter individual variation (Tables 7 and 8).

SALIVA LEVELS OF INTERLEUKIN - 1β AT VARIOUS OBSERVATION PERIODS

- The mean concentration of Interleukin – 1β in Saliva was 186.30 +/- 163.57 Pg/ml at T0, 272.50 +/- 108.16 Pg/ml at T1, and 211.75 +/- 120.09 Pg/ml at T2 (Figure 9).
- The baseline levels for the concentration of IL-1β increased on day 7 after the start of orthodontic treatment and tended to decrease on day 30 after the start of orthodontic treatment when compared to the increased levels seen on day 7.
- Individual variations were very great.
- No statistically significant results were found between the groups because of large amout of inter individual variation (Tables 9 and 10).

FIGURE 8: STANDARD CURVE FOR CONVERSION OF ABSORBENCE (OPTICAL DENSITY) TO PG/ML

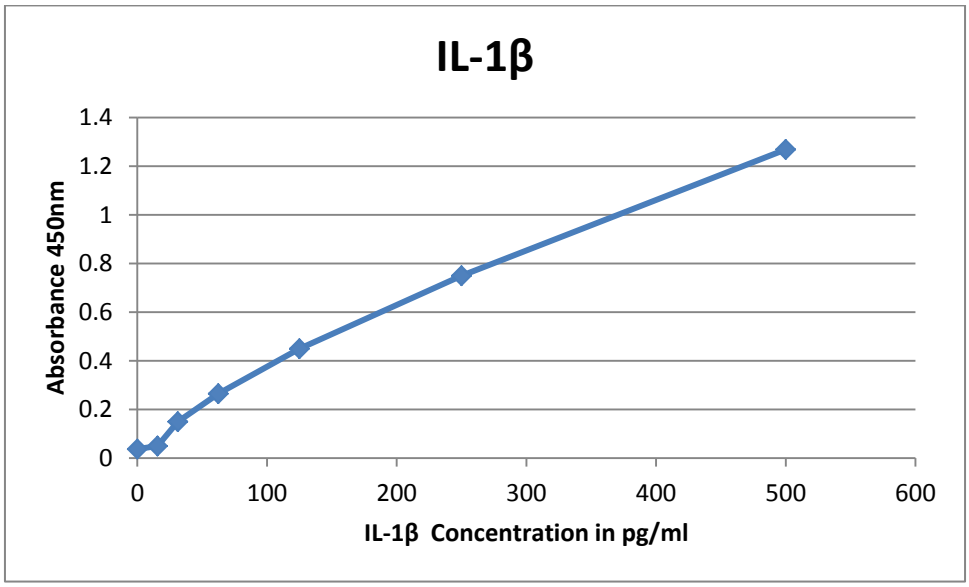


TABLE 2: SALIVA AND GCF LEVELS OF IL - 1β (in pg/ml)

Patient No:	saliva T 0 (Pg/Ml)	GCF T 0 (Pg/ml)	saliva T 1 (Pg/Ml)	GCF T 1 (Pg/ml)	saliva T 2 (Pg/Ml)	GCF T 2 (Pg/ml)
1	126	125	236	238	228	125
2	76	73	> 500*	> 500*	316	356
3	357	211	414	240	408	213
4	75	108	129	167	98	115
5	440	365	> 500*	> 500*	> 500*	> 500*
6	13	3	223	196	159	94
7	59	50	245	343	111	246
8	403	342	> 500*	490	> 500*	346
9	47	14	388	> 500*	77	220
10	267	221	> 500*	346	297	343

T 0 – Pre Treatment

T 1 – 7^{th} day after the start of Orthodontic Treatment

T 2 – 30^{th} day after the start of orthodontic treatment

*Values that are not within the range of specificity of the ELISA kit used (range 0 – 500 pg/ml). These values were excluded in the statistics.

TABLE 3: MEAN VALUES OF IL - 1β IN GCF AND SALIVA AT VARIOUS OBSERVATION PERIODS

		Group			
		Saliva (in pg/ml)		GCF (in pg/ml)	
		Mean	SD	Mean	SD
Obsevation period	T0	186.30	163.57	151.20	129.21
	T1	272.50	108.16	288.57	111.88
	T2	211.75	120.09	228.67	103.37

TABLE 4: T-Test Group Statistics AT T0

	Group	N	Mean	Std. Deviation	Std. Error Mean	F	P
Value T O	Saliva	10	186.30	163.573	51.726	1.849	.191
	GCF	10	151.20	129.211	40.860		

TABLE 5: T-Test Group Statistics AT T1

	Group	N	Mean	Std. Deviation	Std. Error Mean	F	P
Value	Saliva	6	272.50	108.161	44.156	.016	.903
	GCF	7	288.57	111.878	42.286		

TABLE 6: T-Test Group Statistics AT T2

	Group	N	Mean	Std. Deviation	Std. Error Mean	F	p
Value	Saliva	8	211.75	120.085	42.457	.424	.525
	GCF	9	228.67	103.366	34.455		

Table 7: One way ANOVA to compare mean values of IL - 1β in GCF at various observation periods

GCF		Mean	SD	F	P- Value
OBSERVATION PERIOD	T0	151.2	129.21	2.97	0.071
	T1	288.57	111.88		
	T2	228.67	103.37		

Table 8: Tukey HSD Post Hoc Tests for Multiple Comparisons

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	P – Value
T0	T1	-137.37	57.290	.062
	T2	-77.47	53.414	.333
T1	T0	137.37	57.290	.062
	T2	59.90	58.586	.571
T2	T0	77.47	53.414	.333
	T1	-59.90	58.586	.571

Table 9: One way ANOVA to compare mean values of IL - 1β in saliva at various observation periods

SALIVA		Mean	SD	F	P- Value
OBSERVATION PERIOD	T0	186.3	163.57	0.738	0.49
	T1	272.5	108.16		
	T2	211.75	120.09		

Table 10: Tukey HSD Post Hoc Tests for Multiple Comparisons

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	P- Value
T0	T1	-86.20	71.291	.461
	T2	-25.45	65.485	.920
T1	T0	86.20	71.291	.461
	T2	60.75	74.558	.698
T2	T0	25.45	65.485	.920
	T1	-60.75	74.558	.698

FIGURE 8: INTERLEUKIN - 1β LEVELS IN GCF AT VARIOUS OBSERVATION PERIODS

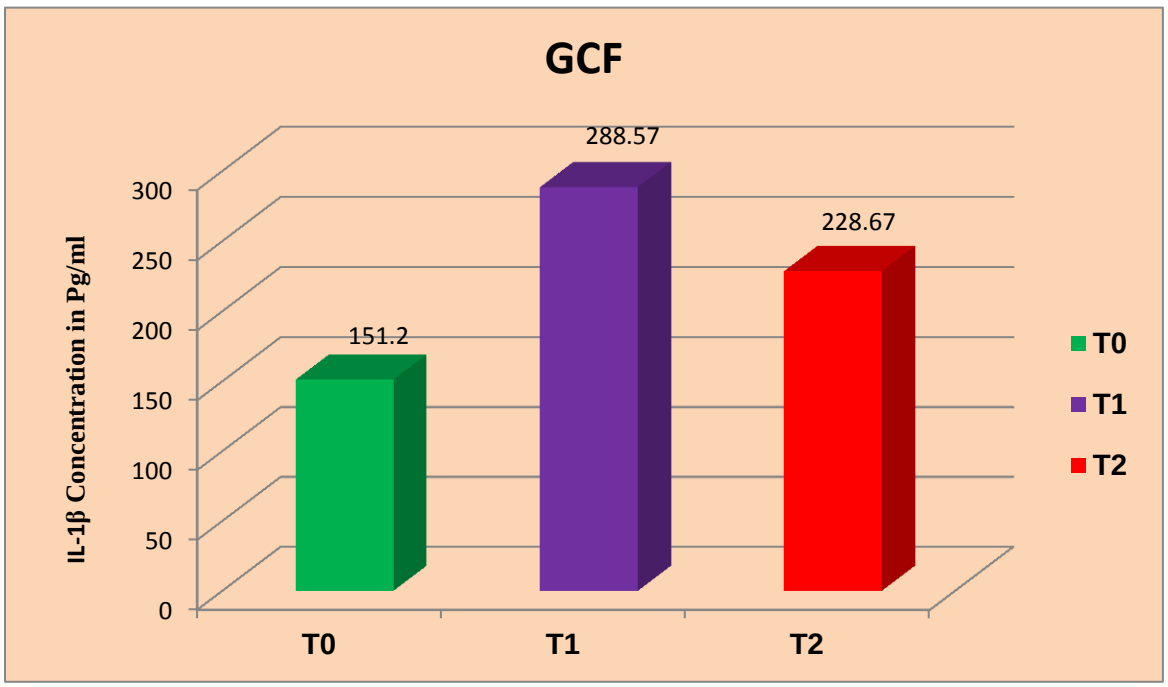


FIGURE 9: INTERLEUKIN - 1β LEVELS IN SALIVA AT VARIOUS OBSERVATION PERIODS

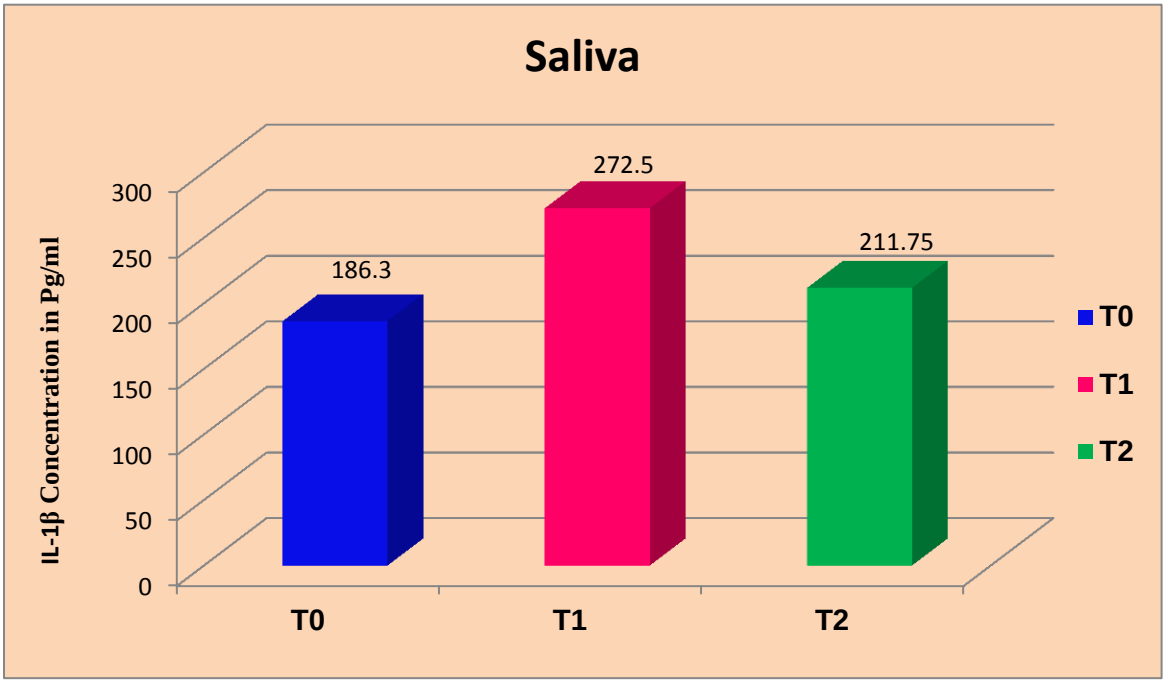
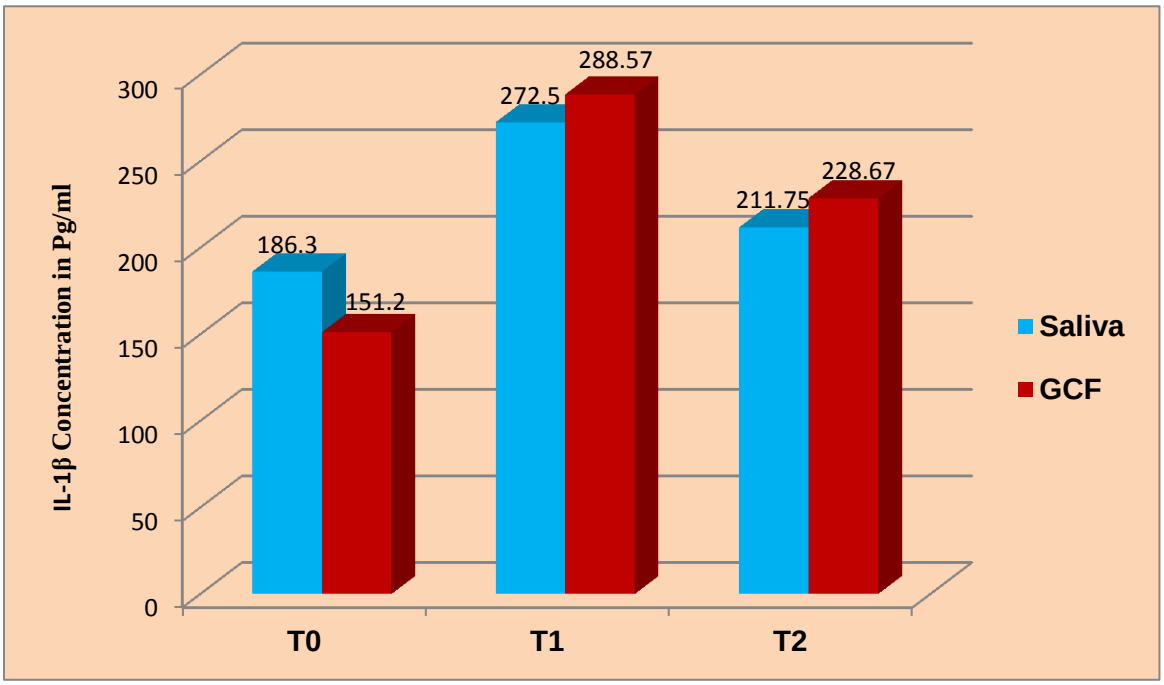


FIGURE 10: INTERLEUKIN - 1β LEVELS IN SALIVA AND GCF AT VARIOUS OBSERVATION PERIODS



Discussion

DISCUSSION

Skeletal bone is actually the result of a dynamic process involving the secretion and resorption of the bone matrix[46,77,78]. **Orthodontic tooth movement** occurs by the remodelling of the alveolar bone as a result of the force that is exerted on the periodontium. A **cell-free hyalinised zone** occurs on the pressure side of the periodontal ligament. Necrosis of this zone is established by osteoclasts that originate from the neighbouring tissues. On the tension side, osteoblasts are responsible in the bone apposition process[27,28,73]. Pro-inflammatory cytokines play important roles in bone resorption as in any root resorption process.[7]

The early phase of orthodontic tooth movement always involves an acute inflammatory response, characterized by periodontal vasodilatation and migration of leucocytes out of the capillaries. These migratory cells produce various cytokines, the local biochemical signal molecules that interact directly or indirectly with the entire population of native paradental cells. Cytokines, acting as paracrines or autocrines, always with other systemic and local signal molecules, evoke the synthesis and secretion of numerous substances by their target cells, including prostaglandins, growth factors, and cytokines. Ultimately, these cells comprise the functional units that remodel the paradental tissues and facilitate tooth movement.[45]

The gingival sulcus was selected as the testing site **in our study** because of its continuity with the PDL and its accessibility within the oral cavity. **Tissue samples** of the PDL or the bone undergoing resorption (or both) would provide a more direct site for measuring changes in cytokines but **cannot be obtained from human subjects**. Thus, cytokine values found in the sulcus provide an indirect measurement of changes in the PDL which result in bone resorption facilitating tooth movement. Localization of cytokines in the PDL of animals can be accomplished by

histologic techniques. The prediction that compression of the PDL in humans could result in the migration of biochemical products into the gingival sulcus is the basis of our experimental design[7,45,109]. This method is a non-invasive procedure and could safely be conducted in Human beings.

It is now an established fact that **gingival crevicular fluid (GCF)** is now considered as an appropriate media for detecting inflammatory changes during Orthodontic Tooth Movement using specified markers[42,45,109]. However the rationale for using GCF on a routine basis in clinical situations is still far-fetched due to various difficulties. **Some of the drawbacks of using GCF include different sampling sites in oral cavity and the differences in sites must be considered. In addition the sampling technique is not easy and a long time is needed for collection of sample[67]. Patient compliance is also a factor when GCF collection is considered.**

Therefore the need of finding an **alternative media** to determine these biological changes has always pondered in the human mind and the first alternative that was thought of was that of the use to find out if saliva could be used as an appropriate alternative.

Saliva is a unique fluid, and interest in it as a diagnostic medium has advanced exponentially in the last 10 years[71]. It has been proposed that saliva could not only be used to help diagnose oral diseases, but as **"the body's mirror"**, it would also have application in the diagnosis of systemic conditions[81]. Saliva harbors a wide spectrum of proteins/peptides, nucleic acids, electrolytes, and hormones that originate from multiple local and systemic sources[40,71]. **Although saliva reflects the body's health and well-being, its use as a diagnostic fluid has been hindered, mainly because of our lack of understanding of the biomolecules present in saliva and**

their relevance to pathological changes, combined with the lack of high-sensitivity detection systems.[71]

Human saliva is produced by glands in various locations in and around the mouth. Three primary glands occur in pairs located symmetrically on both sides of the head: the parotids, the submandibulars, and the sublinguals. In addition to the primary glands, there are also hundreds of smaller glands located in the lips, cheeks, tongue, and palate. The basic role of saliva is to **protect and maintain the integrity** of the upper part of the mucous membrane of the alimentary tract, facilitating important functions.[30,40,71,95]

In general, healthy adults produce 500–1500 mL of saliva per day, at a rate of approximately 0.5 mL/min,[11] but several physiological and pathological conditions can modify saliva production quantitatively and qualitatively. Smell and taste stimulate saliva production and secretion, as do chewing, psychological and hormonal status, drugs, age, hereditary influences, oral hygiene, and physical exercise.[110]

Saliva can be considered as gland-specific saliva and whole saliva. **Gland-specific saliva** can be collected directly from individual salivary glands: parotid, submandibular, sublingual, and minor salivary glands. Secretions from both the submandibular and sublingual salivary glands enter the oral cavity through Wharton's duct, and thus the separate collection of saliva from each of these two glands is difficult. The collection and evaluation of the secretions from the individual salivary glands are primarily useful for the detection of gland-specific pathology, *i.e.*, infection and obstruction.[40,71]

However, **whole saliva is the most frequently studied** when salivary analysis is used. Whole saliva (mixed saliva) is a mixture of oral fluids and includes secretions from both the major and minor salivary glands, in addition to several

constituents of non-salivary origin, such as **gingival crevicular fluid (GCF)**, expectorated bronchial and nasal secretions, serum and blood derivatives from oral wounds, bacteria and bacterial products, viruses and fungi, desquamated epithelial cells, other cellular components, and food debris.[40,66,71]

There are several ways by which serum constituents that are not part of the normal salivary constituents (*i.e.*, drugs and hormones) can reach saliva. Within the salivary glands, transfer mechanisms include intracellular and extracellular routes. The most common intracellular route is passive diffusion, although active transport has also been reported. Ultrafiltration, which occurs through the tight junctions between the cells, is the most common extracellular route. In contrast, a serum molecule reaching saliva by diffusion must cross five barriers: the capillary wall, interstitial space, basal cell membrane of the acinus cell or duct cell, cytoplasm of the acinus or duct cell, and the luminal cell membrane. **Serum constituents are also found in whole saliva as a result of GCF outflow**. Depending on the degree of inflammation in the gingiva, GCF is either a serum transudate or, more commonly, an inflammatory exudates that contains serum constituents.[40]

Part of the rationale for investigating cytokines present in saliva in our study is based on the concept that these mediators find their way into whole saliva through GCF. Since GCF levels of cytokines are elevated during orthodontic tooth movement, it was hypothesized that they would also be elevated in whole saliva during orthodontic tooth movement.

Mandibular incisor crowding is one of the most common problems resolved by orthodontic treatment. Its etiology is multifactorial and no single factor has so far been shown to be the primary cause of the problem. Decrease in dental arch length, growth, maturation and aging of the dentition, mesial drift, soft tissue pressure,

pressure from the back of the dental arch, and tooth morphology have all, at various times, been implicated.[96]

Our study was carried out in **patients with severe mandibular crowding** as assessed by **Little's Irregularity Index(LII)[53].** However it tended to exaggerate cases with severe labiolingual displacements with shortage of arch length[32]. Though it had the above mentioned disadvantage, we had used LII because of its simplicity and the purpose of our study was limited to assessment of changes while leveling a crowded mandibular arch and not on validity of LII.

As early as in ***1839, Harris[31]*** gave the idea that orthodontic tooth movement depends on the resorption and deposition of the bone of the socket. However, it was in the 20th century that the original histological investigation that forms the foundation of our present knowledge was carried out by ***Sandstedt (1904 , 1905)[88,89],*** on dogs. The literature is now extensive for our understanding of the tissue, cellular, and molecular mechanisms involved in orthodontic tooth movement.

One of the major responses to orthodontic tooth movement is the resorption of bone in areas traditionally described as being under "pressure" and its deposition in areas of "tension." This raises the question; how do cells distinguish between tension and compression? When compressive or tensile forces were applied to rabbit tibiae, both resulted in new bone formation. If bone cells cannot distinguish between a tensile and a compressive mechanical stress, how is bone resorption mediated on the "compression" side of the alveolar process during orthodontic tooth movement? At ***The Biology of Tooth Movement conference held in Farmington, Conn., Nov. 5 to 7, 1986***, they suggested that the answer was likely to be found in the field of **cytokine biology**: ~ According to this speculative hypothesis, whether the result is the

formation or resorption of bone depends on (1) the cytokines produced locally by mechanically activated cells; and (2) the functional state of the available target cells[91].

Cytokines that were found to affect bone metabolism, and thereby orthodontic tooth movement, include interleukin 1 (IL-1), interleukin 2 (IL-2) interleukin 3 (IL-3), interleukin 6 (IL-6), interleukin 8 (IL-8), tumor necrosis factor alpha (TNFα), gamma interferon (IFNγ), and osteoclast differentiation factor (ODF). **The most potent among these is IL-1**, which directly stimulates osteoclast function through IL-1 type 1 receptor, expressed by osteoclasts[45].

Secretion of IL-1 is triggered by various stimuli, including neurotransmitters, bacterial products, other cytokines, and **mechanical forces[16].** IL-1 has 2 forms—α and β—that code different genes[17,18]. These interleukins have been reported to have similar biologic actions, systemically and locally. These actions include attracting leukocytes and stimulating fibroblasts, endothelial cells, osteoclasts, and osteoblasts to promote **bone resorption** and inhibit bone formation. Osteoblasts are target cells for IL-1, which in turn conveys messages to osteoclasts to resorb bone.[17,18,45,79]

IL-1 is known to stimulate the proliferation of keratinocytes, fibroblasts, and endothelial cells and to enhance fibroblast synthesis of type I procollagen, collagenase, hyaluronate, fibronectin, and prostaglandin E2 IL-I is, therefore, **a critical component in the homeostasis of periodontal tissues.** However, unrestricted production of IL-1 may lead to tissue damage by enhancement of bone resorption, stimulation of metalloproteinase production, stimulation of plasminogen activator and stimulation of prostaglandin synthesis.

Grieve et al[29] reported that the IL-1β and Prostaglandin E (PGE) levels in human GCF were elevated during the first 24 hours of tooth movement after force

application and returned to baseline in 7 days. ***Uematsu et al***[107] showed similar results regarding ILs, tumor necrosis factor α, and epidermal growth factor. **They assumed that the restored cytokine levels might be due to a lack of force consistency.** These studies applied distalization forces to the teeth and searched for **early responses** to the forces.

The concept of **optimal orthodontic forces** has been discussed since the early 20th century[74,75,93,99,100]. They had claimed that the rate of tooth movement increases with force up to a point, after which the rate decreases or ceases as force levels continue to increase. Proponents would argue that above the optimal force, greater forces prevent the recruitment or differentiation of cells or that the high pressures cause tissue hyalinization, slowing tooth movement and affecting cell-tissue interactions.[28,29,99,100]

Nowadays, **''light continuous forces''** are thought of as physiologically suitable and efficacious[29,73]. In this context, **nickel titanium archwires** have become increasingly popular in recent years because of their ability to release **constant, light forces,** which are considered to improve the efficiency and efficacy of treatment, especially during initial alignment and leveling phases[55]. **We had** used an **0.014" NiTi archwire** as the initial leveling archwire **in our study to provide light continuos forces and tested IL - 1β levels during first 30 days of leveling and aligning of the mandibular arch.**

Emanuela Serra et al[20] stated that **age and sex does not increase enzymatic activity so age and sex differences were not considered in our study.**

So the point to be proven is whether saliva can be used as an alternative to GCF in assessing inflammatory markers during tooth movement.

The **value of GCF in the assessment of the biological state** of deeper-seated tissues of the periodontium lends itself as a source of the biomarkers of specific clinical situations. This is of particular merit in monitoring the efficiency and outcomes of orthodontic treatment, primarily the response of alveolar bone to forced movement.[45]

GCF is a fluid that arises at the gingival margin and can variously be described as a transudate or an exudate[45,109]. Its rate of flow is related to the degree of gingival inflammation, and a rate of 0.05 to 0.20 μl per minute was reported in cases of apparent minimal inflammation. The total fluid flow is between 0.5 and 2.4 ml per day[45]. The fluid has been used to analyze various biochemical markers such as prostaglandin production and the action of various extracellular and intracellular factors, such as IL-1, IL-6, TNF-α, epidermal growth factors, β2 microglobulin, cathepsin, aspartate aminotransferease, alkaline phosphatase, and lactate dehydrogenase.[6,20,21,45,61,70,92,101]

GCF can be collected by variety of procedures providing noninvasive, site specific process, however, the biggest disadvantage, being that it s a time consuming procedure and also the quantity of GCF collected is also very limited. Different methods of collection ranges from the use of platinum loops, filter paper strips, gingival washings and use of micropippetes[42,45,66,109]. Most of the previous studies have used the paper strip for collection of GCF following the procedure described by ***Uematsu et al***[107]. The use of filter paper strips for the collection of GCF is less disturbing to the crevicular epithelium and hence reduces the probability of altering the GCF by excessive contamination with serum. **However using Paper strips one cannot collect predetermined volumes** and assessing the volume requires another

instrument called the **Periotron.** The volume of GCF can be accurately determined between 0.2 and 0.4 pL with the Periotron. However, the **accuracy** of this instrument below 0.1 pL is questionable[37]. **As a result, small errors in volume determination can lead to large errors in estimating the final concentrations when the total volumes collected are small.[48]**

For **collection of predetermined volumes**, **micropippetes** are placed at the gingival crevice and either held at a particular site, or passed back and forth for 10- to 15- minute periods[42]. ***Waddington et al[109]*** in their study on proteoglycans and orthodontic tooth movement stated that **they preferred the use of micropippetes because it has the advantage of ready storage and quantification. We have used micropippetes for collection of GCF in our study based on the advantages as mentioned by *Waddington et al[109]*.** Sampling was performed only on the buccal sides of the tooth to prevent salivary contamination.[7]

On the other hand, Whole saliva can be collected with or without stimulation[40,66,71]. Stimulated saliva is collected by masticatory action (*i.e.*, from a subject chewing on paraffin) or by gustatory stimulation (*i.e.*, application of citric acid on the subject's tongue)[57]. **Stimulation** obviously **affects the quantity of saliva**; however, **the concentrations of some constituents and the pH of the fluid are also affected[40,71]**. **Unstimulated saliva** is collected without exogenous gustatory, masticatory, or mechanical stimulation. The best two ways to collect whole saliva are the draining method, in which saliva is allowed to drip off the lower lip, and the **spitting method**, in which the subject expectorates saliva into a sterile container (Navazesh, 1993)[65]. Salivary composition can also be influenced by the method of collection and the degree of stimulation of salivary flow[40,71]. Hence, **in our study**, we have collected unstimulated **whole saliva** using **the spitting method**.

Our study protocols took into consideration plaque accumulation and gingival condition, so that the effect of bacteria-induced inflammation[25,42] could be diminished.

The results of our study showed that mean concentrations of IL-1β in GCF and saliva on the 7th day (GCF - 288.57 +/- 111.88 Pg/ml, Saliva - 272.50 +/- 108.16 Pg/ml) were significantly higher compared with the baseline (GCF - 151.20 +/- 129.21 Pg/ml, Saliva - 186.30 +/- 163.57 Pg/ml), but this was not statistically significant because of the large inter individual variation. These **findings are in agreement** with the results obtained by ***Basaran et al[7].*** They did a study to test whether interleukine 1β (IL-1β) and tumor necrosis factor–α (TNF-α) levels differ from each other in different treatment levels. In their study, eighteen patients, nine female and nine male (aged 16–19 years; mean 17.4 +/- 1.8 years), had participated. GCF sampling was obtained at baseline, at days 7 and 21 and at the 3rd and 6th month as the leveling of the teeth occurred. Records of the baseline scores for the distalization forces were taken at the 6th month. The results of their study indicated that the baseline levels for the concentration of IL-1β and TNF-α increased on days 7 and 21 of leveling. This increase was more prominent in the concentration of IL-1β but individual variations were very great.

The results of our study showed that IL - 1β levels were highly increased on the 7th day (GCF - 288.57 +/- 111.88 Pg/ml, Saliva - 272.50 +/- 108.16 Pg/ml) **after appliance activation when compared to the baseline levels** (GCF - 151.20 +/- 129.21 Pg/ml, Saliva - 186.30 +/- 163.57 Pg/ml)**, in both GCF and saliva, and they gradually decreased on the 30th day** (GCF - 228.67 +/- 103.37 Pg/ml, Saliva - 211.75 +/- 120.09 Pg/ml) when compared to the increased levels seen on 7th day.

During the earlier stages of tooth movement, **IL-1β** may derive from many periodontal cell types. Thus, the **increase in IL-1β** increase may be associated with undermining resorption following hyalinization induced by heavy forces. IL-1β is thought to be secreted primarily by macrophages, which are involved in the phagocytosis of necrotic tissue and degradation of hyalinized bone and contribute to the repair process. However, macrophage accumulation in compressed areas has been observed in later stages after initiation of tooth movement. It may be possible that IL-1β is released from cell types other than macrophages, such as the osteoblasts, as an immediate response to mechanical stress. IL-1β produced by the osteoblasts leads to production of cAMP, stimulation of PGE production, and triggering of the bone resorption process by the osteoclasts. Altematively, IL-1β can act as a chemotactic factor for the polymorphonuclear neutrophils, associated with the increase in β-glucoronidase observed later in treatment. **At later stages of orthodontic movement**, only osteoclasts and adjacent mononuclear cells in resorption sites within the bone stained distinctly for IL-1β. Perhaps at this stage it is **not possible to detect more subtle alterations in mediator levels that are occurring primarily in the bone[42].** ***Iwasaki et al*** reported that **IL-1β levels fluctuated** with a **28-day cycle** when a continuous orthodontic force was applied[39,42]. **These two reasons might form the basis of our findings of the tendency of IL -1β levels to gradually decrease on the 30th day when compared to the levels seen on 7th day.**

The results of our study showed high amount of inter individual variability in the levels of IL - 1β. Variation in IL -1β level among patients undergoing orthodontic treatment is well documented[3,7,38,39]. *Basaran et al*[7]. in a similar study on assessment of GCF levels of IL - 1β obtained high amount of inter individual variation in IL - 1β levels in their results. Variation in IL-1 level among

patients undergoing orthodontic treatment is well documented as described by ***Al-Qawasmi et al.*** [3]

Saliva results seem to reflect the GCF results in our study, suggesting that probably most teeth in the mouth were subjected to the same stimulus. Although **gingival crevicular fluid** might be a potential candidate for assessing inflammatory markers, there are **certain limiting factors** which include different sampling sites in oral cavity and the differences in sites must be considered. In addition the sampling technique is not easy and a long time is needed for collection of sample[67]. On the contrary, **Saliva** can be collected noninvasively by individuals with modest training, and it offers a cost effective approach for the screening of large populations. There is minimal risk of contracting infections during saliva collection, and saliva can be used in clinically challenging situations, such as obtaining samples from children or handicapped or anxious patients, in whom other methods of sampling could be a difficult act to perform. The collection of the fluid is associated with **fewer compliance problems** as compared with other methods. Saliva contains many enzymes and some inflammatory markers and **no specific laboratory devices are needed for collection of saliva.[40,71]**

These findings are in agreement with the findings of ***Marcaccini et al[58].*** Their study showed that Myeloperoxidase (MPO) activity is highly increased 2 hours after appliance activation, in both GCF and saliva, and that it decreases to baseline levels after 7 days. **Saliva results seemed to reflect the GCF results in their study.**

Results of the current study, show a positive indication that whole saliva could be used as a viable substitute for GCF when used for assessing bone remodeling for patients undergoing orthodontic treatment.

Research regarding the mechanism and evaluation of bone metabolism in orthodontic tooth movement is related to its **potential pharmacologic modulation**. Anti-inflammatory, analgesic, and antipyretic drugs, when prescribed to control the pain and discomfort associated with orthodontic appliance activation and also prescribed for other indications during orthodontic treatment, could decrease the rate of tooth movement[12]. These drugs inhibit cyclooxygenase activity and therefore the synthesis of prostaglandins. Studies of animals treated with prostaglandin inhibitors reported decreased osteoclastic activity[90]. When lipoxygenase inhibitors were used to block leukotriene production, a significant decrease in the rate of tooth movement was observed[64]. In these studies, higher than therapeutic doses, were used for periods of days. However, in clinical practice, these drugs are used at lower doses for only 1 or 2 days after activation.

From another perspective, **local administration of PGE or IL-1β** could **augment the rate of tooth movement**. In fact, this has been observed in both monkeys and human beings after local administration of PGE, with an absence of macroscopic, radiographic, or local side effects[114,115]. Also, local or systemic **deleterious effects** have not been observed after local administration of **IL-1β.**[97]

Furthermore, when an association between clinical measures and measures of tissue remodeling in the GCF and saliva has been established, the latter could be clinically useful to **biologically monitor and predict the outcome of orthodontic treatment.** Thus, **appliance management** could be based on individual tissue response, and the effectiveness of treatment could be improved by **determining the optimum force magnitude** that would provide most rapid tooth movement with the fewest side effects (For example, bone loss, gingival recession, and root resorption).[42]

GCF and saliva analysis may also provide a means of **assessing patient compliance** (For example, to assess if the patient is following proper protocol for palatal expansion). ***Tzannetou et al***[102,103] reported increased levels of IL-1β and βG in patients undergoing palatal expansion. They also stated that stronger forces cause higher levels of inflammatory mediators, and both IL-1β and βG respond to direct and indirect application of mechanical force to teeth.

In addition, the **longitudinal effects of different force delivery systems** on tooth movement, can be investigated by evaluating changes in the GCF and saliva. By such means, it has been suggested that in vivo, nickel-titanium archwires cause relatively constant periodontal tissue perturbation[5], as would be expected of archwires with super-elastic properties[10,62], despite contrasting evidence obtained in vitro.[94]

Alternatively, such analysis may provide a noninvasive method allowing modification of **retention procedures** after orthodontic treatment. The nature and extent of post-treatment relapse following retention has been found to be unpredictable[54]. Since orthodontic tooth movement brings about changes in GCF and saliva composition, the absence of such changes may show that bony changes have been curtailed. Therefore, the point where the **chance of relapse** is minimal can be determined.

Tzannetou et al[102] collected GCF samples from teeth undergoing rapid palatal expansion using filter paper strips. Samples from the mesiopalatal aspects were analyzed for total IL-1β using an enzyme- linked immunosorbent assay (ELISA) method. The researchers observed an early increase in IL-1β levels in response to orthodontic/orthopedic forces. These changes were attributed to mechanical stimulation and not to bacterially induced inflammation, as the Plaque Index remained low throughout the study. **In the post-activation period, increased levels**

of IL-1β were observed as well. This finding may be attributed either to the relapse tendency of the 2 maxillary segments and the bone remodeling around the anchor teeth, or to alterations in palatal and buccal soft tissues.

Variability in the speed of tooth movement between subjects with the same applied force has been noted in humans and animals even between littermates. These findings suggest strongly that individual-specific characteristics are important to the biological responses that result in bone modeling when forces are applied. The ratio of 2 inflammatory mediators involved in bone remodeling, IL-1β and its naturally occurring competitive receptor antagonist, IL-1RA, measured in GCF during tooth movement accounted for up to 60% of the inter subject variation for equivalent stresses. Because predictable tooth movement requires control of bone resorption, the **balance between IL-1β and IL-1RA** synthesis might be important to the bone modeling processes of tooth movement. **Genetic differences are likely to account, in part, for these differences in cytokine secretion and rate of tooth movement for equivalent stresses.**[39]

Clinical manifestation of **External apical root resorption (EARR)** among **orthodontic patients** is highly variable. The IL-1 gene cluster on human chromosome 2q13 includes 3 genes. Two genes (*IL-1A* and *IL-1B*) encode proinflammatory cytokine proteins IL-1α and IL-1β, respectively, and the third gene (*IL-1RN*) encodes a related protein (IL-1ra) that acts as a receptor antagonist. IL-1β has been implicated in bone resorption (catabolic modeling) accompanying orthodontic tooth movement. Variation in IL-1 level among patients undergoing orthodontic treatment is well documented. It is found to correlate with inter individual differences in the amount of tooth translation and might contribute to EARR susceptibility. Such differences might be attributed, in part, to the alleles of the

polymorphic *IL-1B* gene because allele 2 of *IL-1B* has been associated with a 4-fold increase in IL-1β production[3].

However, reservations exist on whether direct extrapolations can be made from the results mentioned to everyday clinical practice. To evaluate the effects of mechanical orthodontic stimuli on GCF flow and composition, research protocols took into consideration plaque accumulation and gingival condition. Thus, the effect of bacteria induced inflammation could be diminished. Nevertheless, **in clinical practice, such an effect cannot be controlled**, as it depends on patient compliance and dental hygiene, as well as mechanical irritation from the band or the cement.[8]

Another limitation of GCF-based studies arises from the inherent variability of the quantities of the components and GCF volume, which is partly attributable to the method of collection used. The standard practice for each tooth under control is gentle washing with water or supragingival plaque removal, isolation with cotton rolls, and gentle drying with an air syringe. This procedure by itself **may disturb the tissues and capillary permeability** (although it is unlikely), resulting in serum influx and influencing GCF volume and components, especially when gingival status is compromised. In addition, **GCF sampling with microcapillary tubules often disrupts the crevicular epithelium and results in contamination of the native GCF with blood and serum.** In addition, collection of standardized volumes can cause a disruption, leading to a serum influx into the crevice and a subsequent dilution of the native GCF by serum.[42]

As a diagnostic medium, **saliva has some disadvantages**. For example, owing to the **diurnal/circadian variations** of certain biomolecules present in saliva, it does not always reliably reflect the concentrations of these molecules in GCF and

serum. Salivary composition **can also be influenced by the method of collection and the degree of stimulation of salivary flow.** Levels of inflammatory markers seen in Saliva can not only be influenced by GCF but also by various other factors like secretion of inflammatory markers by oral epithelial cells, circulatory changes in blood and glandular secretions[40,71,81]. However, this is likely to happen only in the presence of some underlying pathology and not in the case of healthy individuals.

The clinical implications of this study are still far-fetched at this stage, as the tests to evaluate the concentrations of these proteins are currently available only in a research setting. Further research in this field could see a refinement in these tests making them suitable for diagnostic use in a clinical setting. This would enable periodic monitoring of the levels of these cytokines in the GCF or saliva of orthodontic patients**. This sort of assessment would give the orthodontist an insight into the genetic and epigenetic factors affecting tooth movement which cannot be ignored in a clinical setting.**

Summary & Conclusion

SUMMARY AND CONCLUSIONS

This study was undertaken to correlate the findings of presence of Interleukin - 1β in GCF and saliva during initial levelling stage of orthodontic treatment and to substantiate if saliva as a medium could be used as a suitable marker for establishing the expression of IL - 1β.

GCF and Whole Saliva samples were collected from orthodontic patients who had undergone upper and lower first bicuspid extractions. The samples were collected at the following stages:- T 0 – Pre Treatment, T 1 – 7 days after initiation of orthodontic treatment, T 2 - 30 days after initiation of orthodontic treatment. Disposable micropipettes were used for GCF collection. The saliva samples were collected in sterile containers. The collected samples were subjected to an ELISA test to determine the concentrations of IL - 1 β.

- The baseline levels (151.20 +/- 129.21 Pg/ml) for the concentration of IL-1β in GCF increased on day 7 (288.57 +/- 111.88 Pg/ml) after the start of orthodontic treatment. These findings are in agreement with the findings of ***Basaran et al[7].*** The present findings differed from findings of ***Grieve et al[29]*** and ***Uematsu et al[107]*** who showed that IL-1β levels in human GCF were elevated during the first 24 hours of tooth movement and returned to baseline in 7 days when a distalization force was applied. The restored cytokine levels might be due to a lack of force consistency when in particular a distalization force is applied..
- The mean concentration of IL-1β in GCF tended to decrease on day 30 (228.67 +/- 103.37) after the start of orthodontic treatment when compared to

the increased levels seen on day 7 (288.57 +/- 111.88 Pg/ml). These findings are in accordance to the findings of ***Iwasaki et al***[39] who reported that IL-1β levels fluctuated with a 28-day cycle when a continuous orthodontic force was applied.

- The baseline levels for the concentration of IL-1β in saliva (186.30 +/- 163.57 Pg/ml) increased on day 7 (272.50 +/- 108.16 Pg/m) after the start of orthodontic treatment and tended to decrease on day 30 (211.75 +/- 120.09 Pg/ml) after the start of orthodontic treatment.
- Saliva results seem to reflect the GCF results in our study, suggesting that probably most teeth in the mouth were subjected to the same stimulus
- No statistically significant results could be drawn because of high amount of interindividual variability. This finding was the same as those shown in previous studies[7,38,39].

The study design varied from existing studies in following manner:-

- Most of the studies available in the literature regarding the assessment of GCF for IL - 1β during orthodontic tooth movement, have applied heavy distalization forces on tooth. However, in the first stages of orthodontic treatment, low forces are used that do not result in hyalinized zones and indirect resorption. In our study we have assessed levels of IL - 1β in GCF during leveling stages of orthodontic tooth movement in patients with Little's Irregularity Index indicating very severe crowding (score≥10) .
- Most of the previous studies have used paper strips for collection of GCF, following the procedure described by ***Uematsu et al***[107]. One cannot collect

predetermined volumes with this method and assessing the volume requires another instrument called Periotron. We have used micropipettes for collection of GCF in our study based on the advantages as mentioned by ***Waddington et al***[109] which include possibility of collection of predetermined volumes, ready storage and quantification.

- To the best of our knowledge, till date, there is no such study available in literature which is related to our study which involves comparative assessment of IL- 1 β in GCF and Saliva during orthodontic tooth movement.

Results of the current study, show a positive indication that saliva could be used as a viable substitute for GCF when used for assessing bone remodeling for patients undergoing orthodontic treatment.

Saliva has the potential to become a first-line sample of choice to assess orthodontic tooth movement owing to the advancements in detection technologies coupled with combinations of biomolecules with clinical relevance.

Further research in this field could see a refinement in these tests making them suitable for diagnostic use in a clinical setting. This would enable periodic monitoring of the levels of these cytokines in the GCF or saliva of orthodontic patients**. This sort of assessment would give the orthodontist an insight into the genetic and epigenetic factors affecting tooth movement which cannot be ignored in a clinical setting.**

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